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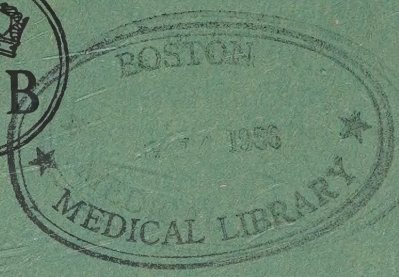

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 2 **October, 1956** *Part 2*

Sarcosporidiosis in Man and Animals

BY

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AND

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No attempt has been made in this review to cite all the very numerous publications on sarcosporidia. For a more complete bibliography reference should be made to Teichmann (1912) and Scott (1943a, b).

Sarcosporidiosis is an infection, most commonly of skeletal muscles, but also of cardiac, lingual, laryngeal, pharyngeal, palatal and oesophageal muscles with organisms of the genus Sarcocystis (Lankester, 1882), (Fig. 1–4). They are seen rarely in lips, skin, intestinal submucosa, dura mater (Scott, 1930). Scott (1943a) described them in the Purkinje fibres of the heart. Until recently sarcocysts were classified as Protozoa; but this view has been challenged, and they may well be fungi (Spindler & Zimmerman, 1945). Although sarcosporidia at times have been claimed to act as causal agents for certain specific animal diseases, e. g. scrapie in sheep (McGowan, 1914), "loco" disease in horses (Watson, 1909) and cattle plague (Beale, 1878), all such assertions have eventually been proved to be erroneous. The parasites probably cause asymptomatic infection in most cases, and their causal role in certain forms of myositis in animals is still doubtful. Usually, sarcosporidial infection of vertebrates is a benign association between host and parasite. The success of this symbiosis is attested to by the wide range of host species, and the world-wide distribution of the infection. A large number of animal species and man are susceptible, including all domesticated and laboratory animals (Fantham & Porter, 1943; Stiles, 1893), reptiles and marsupials (Fantham & Porter,

1916; Darling, 1910), and some wild animals; we have seen sarcocysts* in all of the above and in monkeys, elk (Fig. 4), deer, and armadillo. They are uncommon in white rats. In a survey of 100 consecutive autopsies on white rats in our animal colony, no sarcosporidia were found. We know of no reported cases in Amphibia.

The diagnosis is generally made as an incidental microscopic finding in post mortem material, except in the case of some varieties found in sheep, cattle, buffaloes and pigs, where they may be visible macroscopically.

Economic importance

There is an economic problem involved. In 1953, a little over 1% of all slaughtered cattle rejected by the U.S. Department of Agriculture Bureau of Meat Inspection was on account of sarcosporidiosis (Maddy, 1955). According to the Bureau, most carcasses condemned under the category of "miscellaneous" parasitic diseases are cases of sarcosporidiosis (see Report, Meat Inspection Branch, U. S. Department of Agriculture, Washington, D. C., 1955). Scott (1943b) found that virtually 100% of range sheep in Wyoming were affected, and that the distribution was as wide as that of the sheep industry. Published figures for other countries on the incidence in sheep, swine, and cattle have been as high as 80 and 90% (Bergmann, 1913; Alicata, 1932; Hasselmann, 1923). Crawford (1955) (in a personal communication) mentioned that it was a common cause of rejection of buffalo meat in Ceylon.

Morphology

The mature parasite was first seen and described by Miescher in

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* In the course of a very large number of necropsies on animals we have made a systematic study of muscles from animals, taking pieces from many sites and the oesophagus, heart, diaphragm and tongue. Curiously, we have found sarcocysts most commonly in cats, monkeys and goats, and when present in one muscle they are usually in all sites. Consequently, it is obvious that on many occasions, literally thousands of sarcocysts must be present in an animal's body, and yet do not cause any obvious clinical syndrome. We can hardly believe that there is no pain. Yet we have never found them in the muscles of our rats, and a re-examination of tissues from many hundreds of mice (work done in England by one of us — J. R. M. I.) also indicates that colonies of rodents may be free, and kept free, from infection — an essential requirement in any experimental work.

1843, and as a consequence the psorosperm sacs are often referred to as Miescher's tubes. These can easily be identified in tissue stained by haematoxylin-eosin, where they stand out as blue-stained, encapsulated, granular bodies inside (or amidst) the pink-stained muscle fibres (Fig. 1–6). They are sometimes large enough to be seen by the naked eye as thin white streaks up to 5 cm. long. In sheep, where they may be seen at the lower end of the oesophagus as nodules as large as rice grains, they are prone to be especially large, and were at one time called Balbiana gigantea (Fig. 7a). In the skeletal muscles of buffaloes they are also usually very large and easily seen with the naked eye. It is of interest that their localization ends abruptly at the cardia (Scott, 1930). In wild ducks, apparently, S. rileyi is a common finding in the pectoral muscles and the parasites also tend to be large (Fig. 8).

When removed from muscle fibres, each psorosperm sac is a cylindrical white tube, or cyst, with pointed to rounded ends, a lobulated surface, and a fairly thick cyst wall which often has radial striations. As the parasite matures, fine septa extend from the cyst wall inwards, dividing the tube into separate compartments which are packed with sporoblasts and spores. It is not clear if the cyst wall and septa are derived from the host, the parasite, or both. Scott (1943a) considers that the outer part of the cyst wall is derived from the host, and the inner part and septa from the parasite. On purely morphological grounds we are inclined to agree with him. The sporoblasts are situated towards the periphery and are rounded in shape. The spores known as Rainey's corpuscles (Fig. 9) are banana, sickle or oval-shaped — depending on the degree of maturation — measure 12–16 x 4–9 micra, and contain a large nucleus with a karysome at the rounded end. The pointed end is reputed to have a striated polar capsule, but most observers (Scott, 1943a), including ourselves, have not seen it. Between the nucleus and the pointed end are numerous metachromatic granules. Periodic acid-Schiff staining reveals that the cytoplasm is rich in carbohydrate (glycogen). The maturation of the spores must occur centripetally, for the outer layer of cells is composed of sporoblasts and young spores, and the interior of the cyst contains the mature spores (Craig & Faust, 1943). In some cysts the spores and sporoblasts are so numerous, and so closely packed together, that it is impossible to determine the morphological characteristics of the Rainey's corpuscles, and the sarcocysts simply appear as a dense granular basophilic mass (Fig. 6). The sporoblasts reproduce by binary fission (Negri, 1908; Betegh, 1909; Fiebiger, 1910; Fantham & Porter, 1914). If a cyst is ruptured, and the liberated spores placed in warm saline, they can be seen to move "in a gliding manner on a circumference corresponding to their own curvature for half to two revolutions and then suddenly flop over" (Theobald Smith, 1901). Mature spores resist plasmolysis in saturated sodium chloride solution (Scott, 1943a).

The central (oldest) part of the cyst may degenerate (Fig. 7), leading to necrosis of the entire tube. Mineral material may then be deposited, and leucocytes may be seen in and around the empty cyst (Naidu, 1928), but in some cases we have been unable to recognize the "calcareous focus"

as a sarcocyst (Fig. 10). The cysts lie within a single fibre parallel to the long axis of the muscle fibre (Fig. 2, 4 & 5), simply squeezing aside the striated sarcoplasm, unless they grow larger than the muscle cell, in which case they may occupy interfibrillary tissue. There is rarely any surrounding tissue reaction to the presence of the parasite, and certainly none is seen in the initial stage of invasion, i. e. , as long as the structure of the sarcocyst can be clearly distinguished.

Differentiation from other parasites

A number of other organisms which may reside in striated muscle must be differentiated from sarcosporidia. Toxoplasms are also curved or crescent-shaped parasites with excentric nuclei which can form pseudocysts, but they are smaller than sarcosporidia (2–3 x 4–6 micra). The pseudocysts do not grow as large as sarcocysts, and have neither the striations in the cyst wall nor the septa which are seen in some (but not all) sarcocysts. They are not restricted to skeletal muscle.

Trypanosoma cruzi may appear in muscle as small, thin-walled cysts full of leishmanoid forms of the trypanosomes (Collier et al., 1942; Adams et al. , 1953) (Fig. 13). The parasites measure 1.5–4 micra in diameter and can usually be identified by the large nucleus and kinetoplast. In densely packed cysts, however, where the individual parasites are obscured, it may be difficult to discern intracellular detail. Trypanosomes are also found in blood, and leishmanoid forms in other tissues, particularly in reticulo-endothelial cells.

Biology

Very little is known about the biology of sarcosporidia, and the method of transmission and the life cycle have never been completely resolved. It is even disputed whether they are animal or vegetable. Until recently, they were generally accepted as protozoa of the class Sporozoa, genus Sarcocystis, with a different species from each host. Over 45 have been described so far (Scott, 1930), a few of which are as follows:-

> S. lindemanni (Lindemann, 1868), man; S. muris (Blanchard, 1885), mouse; S. tenella (Railliet, 1886), sheep; S. platydactyli (Bertram, 1892), gecko; S. bertrami (Doflein, 1901), horse; S. blanchardi (Doflein, 1901), cattle; S. tenella bubali (Doflein, 1901), water buffalo; S. hueti (Blanchard, 1885), seal; S. colii (Fantham, 1913), African mouse-bird.
>
> Fibrocystis tarandi (Hawden, 1922) in reindeer and caribou, so-called because the parasites lodge in connective tissue rather than muscle.

Apart from the macroscopically voluminous forms, the species cannot be distinguished morphologically from one another in sections of muscle. There are obvious differences in size, apart from those due to age.

Many authors felt there were fewer species than the above list indicates, and Alexeieff (1913) believed all Sarcosporidia were variants of a single species. Barretto (1940) believed they were a class of Plasmodroma (Doflein, 1901) between Sporozoa and Cnidosporidia.

Possible fungal nature of the organism

Spindler & Zimmerman (1945) claimed to have cultured sarcosporidia for the first time. This gave rise to fresh possibilities. Using cysts isolated from swine, they grew the organisms on a nutrient medium, and, from their protocol, actually fulfilled Koch's postulates by transmission experiments. This work has never been confirmed, but we are not aware of any unreported attempts in this direction. Many pathologists have been sceptical of the validity of these experiments, and it is difficult to reconcile their finding with the very old observation, repeatedly made (Pfeiffer, 1890; Smith, 1901; Koch, 1902; Crawley, 1916; Scott, 1930, 1943a), that viable spores isolated from muscle are motile under certain conditions. Nevertheless, their work deserves more notice, and should be repeated before statements regarding the fungal nature of sarcosporidia prematurely creep into text-books.

Transmission

Smith (1901) was the first to succeed in inducing experimental infection in animals. Using wild mice as a source of infection, he fed infected muscle to animals of the same species, and observed sarcocysts within the muscles in 45 days. The earliest stage within a muscle fibre was described as a small fusiform body measuring 0.132 x 0.02 mm. with a delicate, structureless membrane. In unstained preparations, the contents were hyaline with many refringent granules. Maturation proceeded until, in about 70 days, mature spores were seen. He never found sarcocysts outside muscle fibres.

It has been suggested that neonatal, or congenital, infection may occur, since lambs as young as 6 weeks of age have been found to harbour the parasites, and one of us (J. R. M. I.) has seen them in even younger lambs. Hadlow (1955) has seen sarcocysts in two-week-old calves. Attempts to prove intra-uterine infection were unsuccessful (Bertram, 1890), and Smith (1903) in further, but limited experiments, felt he had excluded these hypothetical possibilities. Congenital infection may, however, be a rare occurrence. Spindler et al. (1946) described it in swine, and Nikolsky (1931) found sarcocysts in a bloated calf foetus. Scott (1943a) says that he saw it as a rare occurrence in sheep.

Although ingestion of infected meat may well be the means of infection in carnivores, it cannot account for the common, sometimes heavy, infection in muscles of herbivores, nor for the rareness of infection in man. Nègre (1907) and Crawley (1916) claimed to have infected mice by feeding faeces of animals which had been eating infected meat. Erdmann (1910, 1914) traced the parasites after they had been ingested by mice; she

described the spores penetrating the mucosal cells of the intestine, multiplying for a few days in the intestinal wall, lymphatics and fat tissue, then disappearing for a time, and finally appearing in the muscle. She hesitated to say that the organisms multiplying in the intestine were derived from the spores which she had fed. Crawley (1916) performed similar experiments. He stated that the spores differentiated into male and female forms in the intestine, and that zygotes were produced, which multiplied in the villi. His plates were unconvincing. Scott (1930) re-examined Crawley's slides, and was unable to see any sexual differentiation of the spores, and concluded that the changes which Crawley had described were of a degenerative nature. Marullaz (1920) reported seeing spores in the mucosa two hours after feeding contaminated food. Mitotic figures were seen soon after the spores entered the lymphatic spaces. The spores then divided, and were transported to the liver and spleen, where their products were found. Daughter spores appeared in the muscle in 44–55 days. Another group of spores became fixed in the intestinal mucosa, and multiplied there, reaching their maximum number in about ten days. Arai (1925) reported somewhat different observations. Two hours after feeding, spores were found along the entire small intestine and the upper part of the colon. After four hours all spores were changed in structure and apparently dying. Soon afterwards they disappeared from the intestines completely. No intracellular stages were seen. In 5–6 hours after feeding, spores were found in the blood, and the plates of this last occurrence are convincing. He was unable to trace the sarcosporidia further than this until they appeared in the muscle. Several other authors (Probst, 1910; Castellani & Sturgess, 1919; Sergent, 1921; Scott, 1930) have reported finding spores in the blood (Fig. 10–12). Scott (1930) stated they might be migrating from the muscle to the intestine where they could develop into a sexual form.

Nègre (1918) described a resistant stage in the intestines of mice 15 days after ingestion of sarcocysts. It had disappeared by the 75th day.

Scott (1920a) reared lambs in a screened, insect-free cage protected against the entry of rodents and other animals. Several attempts to prove congenital infection had failed (Bertram, 1892; Smith, 1905), and as Scott had excluded any role by intermediate insect hosts in the design of his experiments he concluded that infection occurred directly *via* contaminated faeces, and postulated the presence of a sexual, free-living stage in the intestines. Fantham & Porter (1914) suggested that the psorosperm sacs in the muscle rupture, releasing sporoblasts which could invade other fibres; but this has never been verified. A word of caution concerning the preceding observations might be appropriate here. There are innate difficulties in recognizing microscopically minute corpuscular bodies (Fig. 11 & 12) in the alimentary canal, blood, lymphatic spaces or muscular tissue, and in relating them to a different stage of a problematical life cycle, unless these bodies possess indisputable morphological characteristics (*cf. Plasmodium* or *Trypanosoma*). Disagreement among authors is such that their descriptions are unconvincing, especially when clear illustrations are lacking. Spindler, Zimmerman and Jacquette have done much to clarify

this problem of how infection is transmitted. Using several animals they showed that feeding infected pork alone was not enough to produce infection consistently, but that if faeces and urine were collected from sarcocyst-fed animals, infection could be accomplished with excreta eliminated 15 or more days after the consumption of infected meat. They point out that since Smith (1901), and Darling (1902), probably took no precautions to prevent coprophagia by their animals, infection in their experiments may well have been via contaminated excreta. They also describe definite clinical signs of vomiting, diarrhoea, laboured breathing, and staggering gait in some pigs fed large numbers of sarcocysts, and less marked reactions in pigs which had a lighter infection. Macroscopically, they saw pale, swollen kidneys and hyperaemia of the gastro-intestinal tract. Dogs, rats and cats showed much milder clinical pictures. These responses, since they were so acute, may well have been due to the endotoxin (sarcocystin). No histological studies are described. In their system, then, infection would be transmitted primarily by ingestion of infected faeces — in man and in animals — and might account for the seasonal infection described by Scott (1919).

It seems to us that the evidence is strong to support the contention that infection is via the alimentary tract by means of food contaminated with faeces and urine, and may well follow the schema of Spindler, Zimmerman and Jacquette*. What happens thereafter — between the time the sarcocysts enter the host's mouth, and the time they are first seen in the muscle fibres — is not known with any degree of certainty.

Darling (1910) took sarcocysts from adult opossums and inserted them into the thigh muscles of guinea-pigs. In 2 of 6 guinea-pigs, he saw what he called Sarcosporidia, but the parasites were smaller and of somewhat different morphology than those in the original host. In examining other animals, he noticed that the cysts and spores were consistently smaller than in some animals (man, sloth, opossum, hawk) than in others (sheep, cat, rat). He felt that the size and morphology of the sarcosporidia were related to the host species, rather than to the species of sarcosporidia, and that Sarcosporidia were Neosporidia, whose natural hosts are commonly invertebrates. In his schema, infection of vertebrates was an accident of nature, in which an animal became infected by ingestion of spores deposited by insects on herbage and pastures. In the vertebrates, which then would be unnatural hosts, the parasites enter a blind alley of development, for, although they be in tissue hospitable for some period of their maturation, there is no means of exit for continuation of the life cycle. Nègre (1907) concurred with this interpretation. Supporting evidence was supplied by Scott (1919) and Bergmann (1913) who reported a seasonal variation in infection, with greater severity in the spring and summer.

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* In our experiments we have been unable to transmit infection by feeding infected ox heart to rats, and the faeces of the latter to other rats.

This hypothesis has, however, met several objections. Scott (1920a, 1930) tested Darling's idea, even to the extent of feeding to his animals a variety of insects of the neighbourhood which were the only possible insect hosts, and could find no evidence to support the existence of an intermediate insect host as a factor in infection. Darling's work has, however, led to some interesting studies by Kean & Grocott (1945). These latter two authors reviewed the reported cases of human sarcosporidiosis, and noted a distinct variation in the size and morphology of the spores and cysts. There seemed to be two groups, one with large, classical Miescher's tubes, and a second with smaller organisms and a somewhat atypical morphology. Darling, in his experiments on guinea-pigs, claimed that the animals used were naturally free from sarcocysts. Kean and Grocott found what Darling and Nègre had classified as the smaller type of sarcocysts in 5 of 60 guinea-pigs. They also found a cyst in the brain of one of two guinea-pigs on which complete autopsies were performed. They suggested that the hypothesis of Darling and Nègre was unsubstantiated, and that the smaller organisms were either toxoplasms or some other parasitic species. Since our only criterion for the diagnosis of sarcosporidiosis is morphological, and anatomical location helps considerably, and as the work on toxoplasmosis is relatively recent, their arguments merit serious consideration. One reported case of sarcosporidiosis (Hertig, 1934) was later described by Pinkerton & Weinman (1940) as toxoplasmosis. We feel sure that other cases of toxoplasmosis in animals and man may have been called sarcosporidiosis and vice versa. It must not be forgotten, however, that at least some of the morphological variation reported may be a result of the age of the cyst examined and of local tissue factors.

Pathogenicity

A heterogeneous variety of conditions in domesticated animals, such as eosinophilic myositis (see later remarks), watery flesh, myositis and perimyositis with obliterating endarteritis, hypertrophy of the perimysium, myocarditis, endocarditis and pallor of the muscle, have been thought at various times to be caused by sarcosporidia (Scott, 1930), but the aetiological role of the parasites in causing specific disease of muscle remains as obscure as when Miescher found them.

It is notable that Smith's (1901) mice, when heavily infected, showed definite signs of "slowness and unthriftiness". Scott (1930) made similar observations. Scott also felt that heavy infestation in sheep could cause death. Yet, such clinical signs are really non-specific, and might be very unreliable, in view of the protean variety of diseases which affect mice and cause "unthriftiness". In the muscle of his mice Smith noted multiplication of muscle nuclei, foci of leucocytic infiltration, and proliferation of the perimysium internum and the sheaths of the small interfibrillary blood vessels. The affected fibres and the tissue components immediately adjacent were essentially normal.

Pfeiffer (1890), Kasparek (1895), Laveran & Mesnil (1899b) and Sabrazes & Muratet (1911), described an endotoxin which was called

sarcocystin. Sato (1926), who called it sarcosporidiotoxin, studied it again in some detail. He used dried extracts of S. blanchardi, which he found to occur in 56% of Formosan buffaloes. The toxin was destroyed by acids, by CO_2, and by heating at 50° C. for 30 minutes, but it was resistant to alkali. The rabbit was the animal most susceptible to the toxin. The toxin was inert when swallowed or injected into the bronchi, "weak" when injected intracerebrally, still "weaker" intraperitoneally, but very potent intravenously. The intravenous m.l.d. for rabbits was reported to be 0.005 mg./kg. The clinical picture included excitement, "paralytic collapse", severe diarrhoea, and death. From post mortem material, Sato concluded that the toxin had effects (unspecified) on the liver, adrenals, alimentary tract and heart. When the toxin was "brought into contact" with any of these tissues in vitro, the "toxicity disappeared". Rabbits could be immunized, but immune serum, although it neutralized the toxin in vitro, did not always do so completely in vivo. It is difficult to evaluate this work, although it was obviously carefully done, because Sato wrote in Japanese and the terms are not too clear in the English translation. The details of Sato's work have never been confirmed, although Rievel & Behrens (1903) reported extracting a neurotoxin from sarcosporidia from the llama. Teichmann & Braun (1911) and Cominotti (1913) state that the toxin is bound to the lipids of the central nervous system, and Cominotti found that repeated injections into guinea-pigs resulted in anaphylaxis.

The presence of sarcocystin, together with the severe infection which sometimes occurs, has been alluded to repeatedly in speculations as to what disease states, if any, are caused by sarcosporidia. It should be emphasized, however, that the toxin is an endotoxin, and hence could be active only when the parasite is destroyed, i. e. , either in the intestine at the time of entrance into the host, or when a cyst ruptures or dies. It is tempting to consider that the acute "toxaemia" in animals observed by Spindler et al. (1946), which developed after ingestion of sarcocyst-infected flesh, was due to the destruction of Rainey's corpuscles in the intestine. It has, however, never been proved that sarcocystin plays any role in natural, or experimental, infection.

It is evident, from what we have said above, that the pathogenic role, if any, of sarcosporidia is still unknown. Some authors, among whom was the eminent Virchow in 1866, have incriminated the parasite in a variety of diseases and anatomical lesions. At least one case report of sarcosporidiosis in a cow (Bowler, 1948) makes a rather convincing claim that massive infection with sarcocysts was the cause of death. Claims have been made that the death of the parasites precipitated a chronic focal, or disseminated, myositis with destruction of fibres, and marked inflammation with great preponderance of eosinophile leucocytes — myositis sarcosporidica chronica (Nieberle & Cohrs, 1952) (Fig. 14, 15, 16, 17). This may be of some significance. In the U. S. A. , as well as in Europe (where it was first described), focal and disseminated forms of eosinophilic myositis (Fig. 16) have been diagnosed in cattle and pigs, and, rarely, in dogs in Europe, Great Britain and America (Winter & Stephenson, 1952; Whitney, 1955). In dogs, the muscles of mastication seem to be a predilection site,

and some cases have been suspected clinically. Although sarcosporidia have never been indisputably shown to be involved in the aetiology of the eosinophilic myopathies, they still have not been totally excluded as a possible cause. The chronic (also eosinophilic) myositis, which has been definitely ascribed by others to be associated with the presence of sarcosporidia, may be only a late stage manifestation of parasitization of muscle fibres; in fact, it has been said to occur only after the parasites are dead and calcified, but then there may be inherent difficulties in recognizing the sarcosporidia (Fig. 10). The observations of Frenkel (1955), on Besnoitia, are of interest in this regard. He found that there is no tissue reaction to the presence of encysted organisms, but if the cyst ruptures, there is a definite inflammatory response. Scott (1943a) considers that sarcocysts do rupture, releasing spores into the blood stream. It is tempting to draw an analogy between the pathogenesis of the lesions associated with these organisms, viz. sarcosporidia and Besnoitia. Lorenz (1904) described a human case of dermatomyositis associated with small, sarcosporidia-like forms in the muscle, which healed spontaneously. Such a coincidence must be a rarity, for Lorenz saw only one case and it has never been reported since. Awad & Lainson (1954) state that sarcosporidia and toxoplasms cross-react in the dye-dilution test. This has not been confirmed.

Infection in man

There have been, thus far, 12 reported human cases of sarcosporidiosis. The incidence is probably higher than this figure would indicate, for routine microscopic examination of skeletal muscle and other muscle is not carried out in many human pathology institutes. Nine cases have been diagnosed at autopsy and three by biopsy specimens from patients: Baraban & St. Rémy (1894); Vuillemin (1902); Manifold (1924); Lambert (1927); Bonne & Soewandi (1929); Darling (1909); Arai (1948); Feng (1932); Price (1932); Hewitt (1933); Gilmore et al. (1942); Naidu (1928); Darling (1920); Vasudevan (1927). In all cases, the diagnosis was an incidental finding unrelated to the nature of the primary disease, or the ultimate outcome. The two cases of Arai (1948) are of interest in that the patients died of trichinosis. The sarcocysts may well have been acquired from the same source as the trichinae. We have illustrated the case of a cat (Fig. 1) with the same co-existing lesions. We have been privileged to examine, and illustrate, one human case in a 30-year-old soldier who had served in the Pacific, who died of tuberculosis (case of Dr. E. Barron, Vet. Admin. Hospital, Kecoughtan, W. Va.). The photograph may show some of the intrinsic difficulties in identification, and the lack of tissue reaction; the parasite was diagnosed by some as Toxoplasma.

CONCLUSIONS

Our lack of knowledge about this ubiquitous parasite regarding hosts and distribution may well be due to its pathogenic inactivity, since it is largely the aesthetic sense of man which makes it an economic problem in domesticated animals — which he must eat. This lack of pathogenicity has excited the curiosity of pathologists, but little enthusiasm for organized

study of the problem. In fact, with the exception of Spindler & Zimmerman's work, there is virtually nothing new since Scott wrote his excellent review in 1930. Although this widely distributed organism is so successful an invader of animals and man, there are several gaps in our knowledge of its life-cycle, of the clinico-pathological effects on its hosts, and of its place in taxonomy, all of which offer opportunity for research in an interesting biological problem.

We conclude by quoting from Scott's review: "Many contradictory opinions have been expressed..., divergent results ... obtained ... erroneous conclusions drawn, frequently from inadequate data." "In scientific procedure there is the need of rigidly accurate observations, carefully planned experiment, and logical reasoning in drawing conclusions. A critical study of the development of knowledge of the Sarcosporidia reveals that ... these principles have ... been violated."

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ILLUSTRATIONS

Fig. 1. Cat, tongue. Two sarcocysts and one trichina larva. No tissue reaction. Haematoxylin-eosin. x 120.

Fig. 2. Cow, tongue. Two sarcocysts (S. blanchardi) in longitudinal section, each within a single fibre, although this is not apparent at this magnification; no reaction. Haematoxylin-eosin. x 120.

Fig. 3. Elk, tongue. Large sarcocysts; no reaction. Haematoxylin-eosin. x 110. (Courtesy of Dr. W. Hadlow, Rocky Mountain Laboratory, Montana).

Fig. 4. Cow, heart. A single sarcocyst within fibre. Haematoxylin-eosin. x 120.

Fig. 5. Man, 30 years old. Pectoral muscle. Accidental finding. Sarcocystis lindemanni originally diagnosed as toxoplasma. Haematoxylin-eosin. x 80. (Courtesy of Dr. E. Barron, Veterans Administration Hospital, Kecoughtan, W. Virginia).

Fig. 6. Cow, heart. Sarcocyst directly adjacent to the lumen (above) of a vein; suggests the possibility of rupture into the circulation and dissemination of spores. Haematoxylin-eosin. x 1200.

Fig. 7. Sheep, lower end of oesophagus. S. tenella, huge cysts with central degeneration, showing why it was at one time called Balbiana gigantea. Haematoxylin-eosin. x circa 6. (Courtesy of Dr. C. L. Davis, Bureau of Animal Industry, Washington, D. C.).

Fig. 8. Wild duck, breast muscle. A number of sarcocysts (S. rileyi); no reaction; apparently a common finding in wild ducks. Haematoxylin-eosin. x circa 6. (Courtesy of Dr. C. L. Davis, Bureau of Animal Industry, Washington, D. C.).

Fig. 9. Goat, heart. A young sarcocyst without septa, showing banana-shaped spores and oval to round sporoblasts. Other morphological details are not discernible. No reaction. Haematoxylin-eosin. x 1200.

Fig. 10. Rabbit, diaphragm. The skeletal muscles and diaphragm were speckled with white spots visible to the naked eye, some of which were calcified. In the absence of typical sarcocysts it would be difficult to recognize such calcified bodies as dead sarcocysts. An identical picture in Adams et al. is labelled trichinosis. Haematoxylin-eosin. x 110.

Fig. 11. Rabbit, skeletal muscle. Two nucleated bodies each within a fibre. To illustrate difficulty of recognizing parasite on morphological grounds alone. Haematoxylin-eosin. x 150.

Fig. 12. Rabbit, skeletal muscle. Numerous unidentified minute circular bodies within a fibre — young sarcocyst spores? See remarks for Fig. 11. Haematoxylin-eosin. x 110.

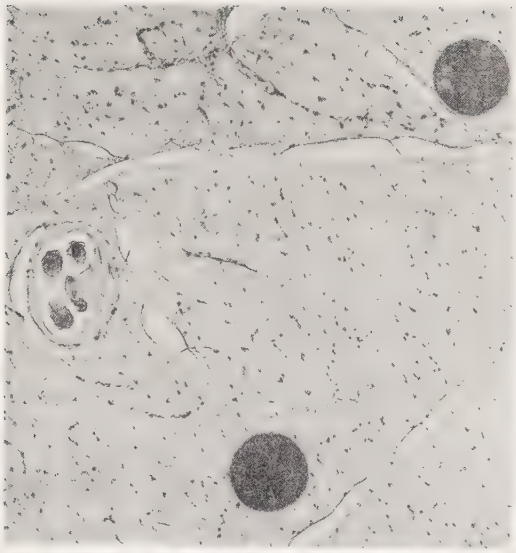
Fig. 13. Experimental Chagas' disease. (T. cruzi infection). Mouse. Acute myocarditis and myositis. Leishmanoid forms occur in muscle fibres and under the low power they may be confused with Toxoplasma and Sarcocystis. Haematoxylin-eosin. x 1200. (See Collier et al., 1942).

Fig. 14. Cow, skeletal muscle. So-called chronic sarcosporidial myositis with central calcified mass, surrounding necrosis and marked inflammatory reaction in which there are many eosinophile leucocytes; figured for years in German literature as a pathological entity. It is difficult to be sure that the central body is an old degenerated sarcocyst. Haematoxylin-eosin. x 110.

Fig. 15. Cow, skeletal muscle. Same section as Fig. 14 but in an area without "sarcocysts" and away from the lesion shown. Fibrosis and leucocytic (mainly eosinophilic) infiltration. In the absence of a lesion such as that seen in Fig. 14, the diagnosis of sarcocystic inflammation would not be contemplated. Haematoxylin-eosin. x 110.

Fig. 16. Cow, skeletal muscle. Eosinophilic myositis with atrophy, degeneration of the fibres, and marked inflammatory infiltrations in which about 90% of the cells are eosinophiles. (Case from New Zealand, courtesy of Mr. W. J. Hartley, M. R. C. V. S., Wallaceville, N. Z.). Haematoxylin-eosin. x 110.

Fig. 17. Cow, skeletal muscle. Eosinophilic myositis, reputed chronic stage of lesion seen in Fig. 16, in which fibrosis is intense. Thickened arteries are prominent. Eosinophiles are still present, but sarcocysts are absent. Haematoxylin-eosin. x 110.



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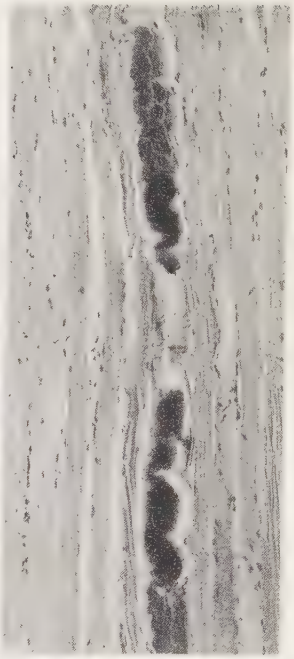

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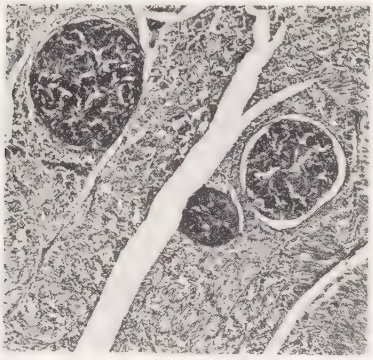

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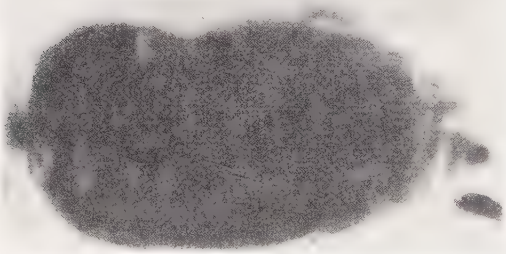

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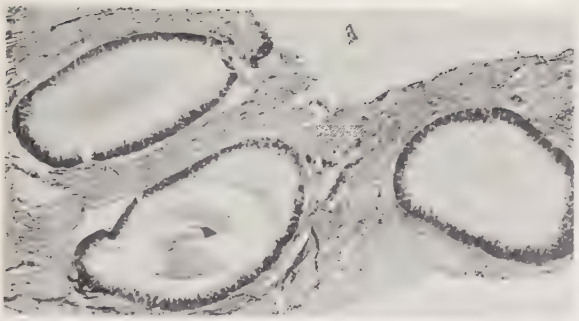

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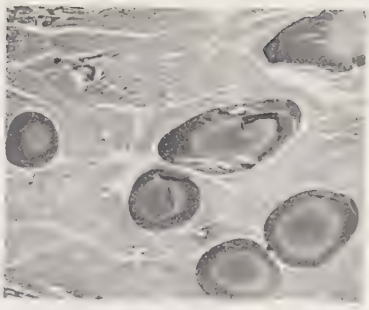

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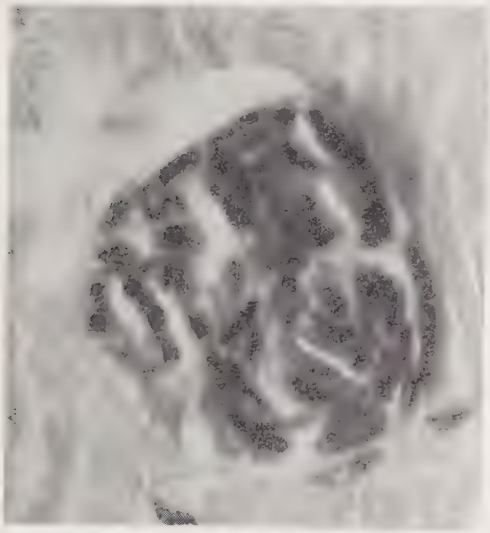

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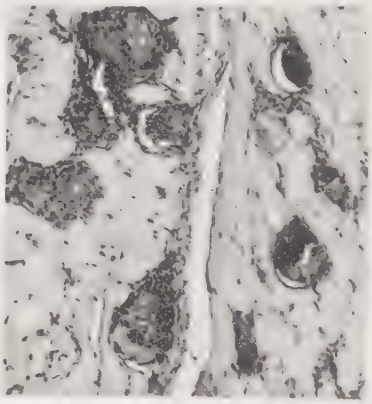

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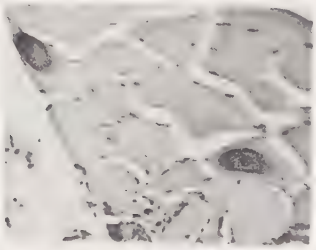

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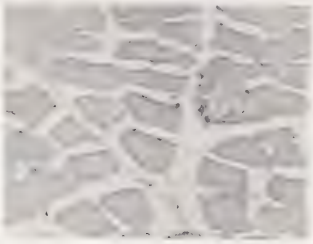

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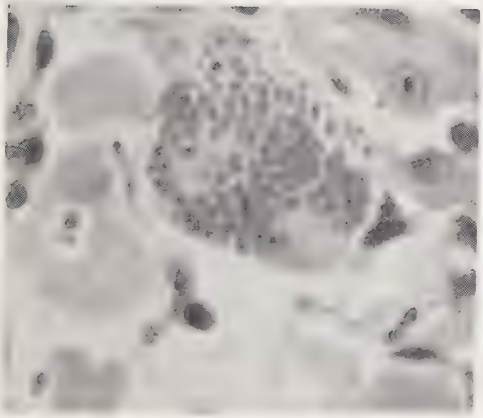

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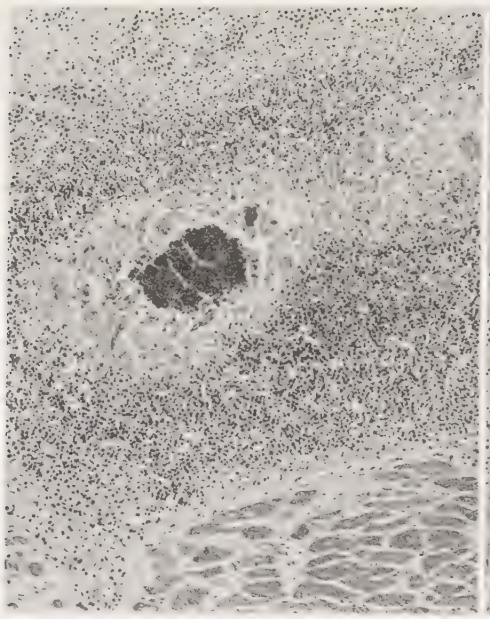

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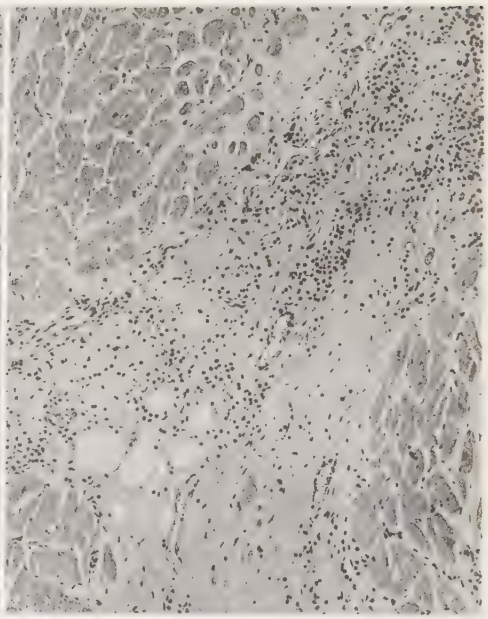

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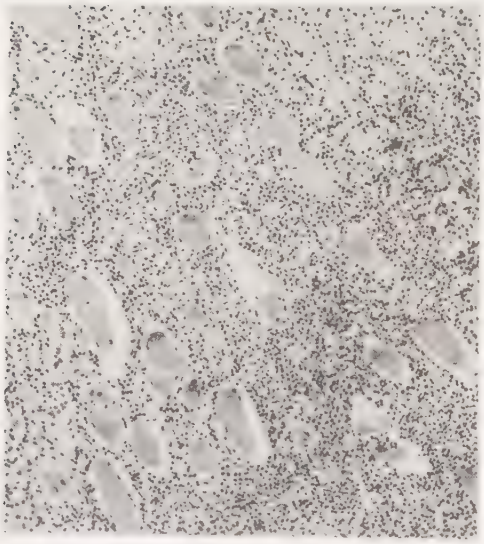

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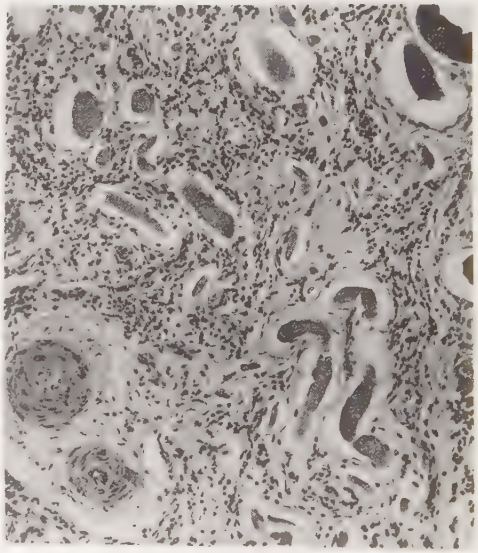

17

Piglet Anaemia

A REVIEW OF THE LITERATURE

BY

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Introduction

Piglet anaemia is a disease with a very recent history. In 1891 Braasch described symptoms and lesions of a chlorosis in young pigs which was complicated by cervical abscess formation. He considered that the disease was brought about by the unnatural methods of pig husbandry being introduced at that time and observed that the condition could be prevented by a supply of earth or green food.

Thirty years later the disease was comprehensively described at the Rowett Institute in Scotland (McGowan & Crichton, 1923; McGowan, 1924; McGowan & Crichton, 1924). These workers described the clinical and pathological signs of the condition and reported that it could be prevented, and early cases cured, by the addition of crude ferric oxide to the nursing sows' rations. They concluded that the disease was due to a deficiency of iron, which was overcome not by way of the sows' milk, but by the piglets eating the iron-rich food splashes made by the sows when feeding.

Incidence

The disease has been reported from Great Britain, Canada, the United States and Denmark (McGowan & Crichton, 1923; Schofield, 1930; Doyle et al., 1927; Adsersen, 1932) and probably occurs wherever intensive systems of pig husbandry are practised. Occurrence seems to be principally dependent on management and climate, and possibly also on genetic factors. The reported incidence in pigs reared on grass, or with access to soil or in pigs receiving satisfactory prophylactic treatment is very low, but cases do occur (Moe et al., 1935). Unless precautions are taken, piglet anaemia or lowered haemoglobin levels will occur frequently in pigs reared in concrete or other solid floored pens. Under such conditions whole litters have often been affected and have died (McGowan & Crichton, 1923), and in the literature figures for the incidence of the disease range from 51% (Doyle et al., 1927) to 91% (Kernkamp, 1935). Morbidity is likely to be greatest when a high degree of cleanliness is maintained in the piggery, because this restricts the sources of iron, and also in herds with high weaning weights, for rapidly growing pigs are most susceptible (Hamilton et al., 1933). Many authors have noted an increased incidence and severity of piglet anaemia in pigs born between late autumn and early

spring (Doyle et al., 1927; Schofield, 1930; Graham & Thorp, 1932; Hamilton et al., 1933). Variation in the actual months reported can be explained by the different climates of the countries concerned. At no season, and under no experimental or observed conditions, has morbidity in a pig population been reported to reach 100%; this may be due to the presence of "resistant" litters: the hereditary background of these is discussed under aetiology.

Economic importance

The mortality of pigs affected with anaemia has ranged from 9% (Kernkamp, 1932) to 60% (Doyle et al., 1927). The bacteriological findings of Adsersen (1932) revealed that anaemic pigs are susceptible to other diseases, especially infectious diseases, and McGowan (1924) and Doyle et al. (1927) found that stunted growth with cirrhotic livers frequently followed recovery. Adsersen (1932) and Jespersen & Olsen (1939), but not Schwarte (1939), found that low haemoglobin levels as well as anaemia reduced growth rate, and Barber et al. (1955b) found that food consumption was also reduced. Prophylactic and therapeutic treatment takes time and adds to production and labour costs. There is no direct evidence of the agronomic importance of the condition.

Aetiology

It is apparent from the literature that piglet anaemia is a man-made disease associated with the introduction of concrete-floored pig houses, and it is an interesting side-light on pig husbandry that Braasch, in what is now part of Denmark, noted an adverse effect of this innovation in the nineteenth century, but 30 years were to elapse before it was seen in Britain and America. McGowan & Crichton (1923), Schofield (1930) and ultimately Doyle (1932) ascribed the disease to iron deficiency brought about by the rearing of pigs in brick and concrete structures. Under these circumstances, the only sources of iron available to piglets after birth are the milk, food splashes or dung of their dam, and such dirt as may accumulate in the sty. It has been known for a long time that milk is a poor source of iron, and Venn et al. (1947) were unable to demonstrate more than 280 microgrammes of the element in 100 g. of sow's milk, even when supplementary iron was given orally. The amounts available from the other three sources diminish in proportion to the degree of cleanliness maintained by the pig keeper. It is noteworthy that McGowan supplied iron to piglets by way of splashes of their dam's food, apparently considering this method more rational than to allow them access to grass or earth.

For a long time the theory of iron reserves in the liver of the new-born to cover the suckling period (Bunge, 1892) attracted the attention of many workers studying piglet anaemia. There are several reports on the administration of iron to pregnant sows in an attempt to build up foetal iron reserves, (Hamilton et al., 1930; Foot & Thompson, 1938; Venn et al., 1947), but all were unsuccessful. The most determined of these was

by Hart et al. (1930), who supplemented the diet of gilts with iron and copper from weaning age to parturition, but even this conferred no benefit on their litters. In each case, however, the iron supplements were given orally, and the lack of success may be attributed to the peculiarities of iron absorption from the gut, but it is possible that the sows' liver iron was increased. No analyses were made to clarify this point, and it would appear unlikely that the rate of iron absorption in the sow increases during pregnancy, especially as an anaemia of pregnancy corresponding to that seen in human medicine has not been reported in swine.

It remains to be seen whether parenterally administered iron would influence the iron content of the foetuses.

Venn et al. (1947) computed the piglet's requirements of iron to be 7 mg. daily during the first three weeks of life, if the amount of iron per unit of body weight found at birth was to be maintained during growth. The liver reserve at birth was a negligible source of iron for haemoglobin formation. Even under conditions which provoked anaemia, piglets obtained 3.5 mg. of iron daily. To maintain normal health a similar quantity would have to be provided during the five weeks which Barber et al. (1955a) found to elapse before piglets consumed appreciable quantities of creep food. Under natural conditions this additional iron would be available from the soil. Venn et al. found 513 mg. of iron in the gut washings of a piglet reared at pasture. Brownlie (1955) considered that if sufficient iron was available following anaemia there was a considerable ability to regenerate haemoglobin rapidly. He suggested that most creep foods contained insufficient iron for this purpose and that the iron content of these foods should exceed 124 p. p. m.

Hastings (1931), who probably confused cause and effect, considered that the disease was due to lack of exercise, leading to too rapid growth and obesity. Fulton (1932) and Graham & Thorp (1932) showed that exercise was not a factor in this condition, but it has frequently been noted that rapidly growing pigs are more prone to anaemia (McGowan & Crichton, 1924; Schofield, 1930; Hamilton et al., 1933), while Schofield considered that both intra- and extra-uterine growth were important factors. As Venn et al. (1947) have shown that the absolute iron content of new-born pigs does not vary according to their size, it follows that, under conditions of iron insufficiency, large pigs will be at a greater disadvantage than small ones, and will be more susceptible to the disease.

Because Doyle et al. (1927) failed to prevent anaemia by adding ferrous lactate to the food offered to sucking pigs, they considered that the disease was due to a deficiency of another factor which the pigs obtained when out of doors. This temporarily diverted attention from iron to the effects of various light waves (Mathews et al., 1929; Craig, 1930) and sunshine (Fulton, 1932). These studies were unproductive, and later Doyle (1932) reported that the factor in outside conditions was actually iron in the soil. Nevertheless Jespersen & Olsen (1939) found higher haemoglobin levels in young pigs in summer than in winter, and they correlated growth

rates with haemoglobin levels. The Shinfield workers also reported better growth rates in summer-reared pigs, but they considered soil to be a more important factor than sunlight in haemoglobin synthesis (Braude, 1949; Barber et al., 1953). There is general agreement in the literature that the incidence and the severity of the disease increase during the winter months. This might be accounted for by lowered temperatures or by reduced sunlight. The work of Jespersen & Olsen (1939) suggests that the latter may be responsible, but Adsersen (1932) was able to correlate mortality with minimum air temperatures. The papers of Howie et al. (1949) and Naftalin & Howie (1949) also suggest that low temperatures, coupled with damp environment, are involved: but although the pathology of the condition which they described closely simulated that of piglet anaemia, the limited haematological data presented does not permit an unequivocal diagnosis.

There are several reports of litters which failed to become anaemic under conditions calculated to provoke anaemia (Schofield, 1930; Doyle, 1932; Adsersen, 1932; Moe et al., 1935). Schofield and Adsersen considered that this resistance might have a genetic origin, whilst Doyle postulated that such pigs were resistant because they had high liver reserves of iron. Jespersen & Olsen (1939) found that certain strains of sows produced piglets with high haemoglobin levels whilst others produced piglets with low haemoglobin levels. Under conditions provoking anaemia, the difference between these strains was distinctly marked, the latter having a high mortality and a low growth rate. The genetic basis of this observation was not explained. It is possible that in some instances, at least, so-called resistance has been reported when piglets have been insufficiently restricted and were able to obtain their requirements of iron. In other cases it is interesting to speculate on the mechanism of the resistance. In view of the findings of Venn et al. (1947) on the iron content of new-born pigs, Doyle's (1932) hypothesis is probably incorrect, but it seems unlikely that an inherited characteristic affecting iron requirements for haemoglobin formation is responsible.

Elvehjem et al. (1932) showed that the pig, like other species, requires copper for the synthesis of haemoglobin. Their work was confirmed and extended by Gubler et al. (1952) who found that iron metabolism was affected by copper deficiency. Brooksbank's (1954) report of field cases of piglet anaemia due to copper deficiency seems to be the only one in the literature. In an early experiment (Hart et al., 1930) pigs obtained no benefit from the administration of copper because the experimental conditions were not closely restricted, and the supposedly copper deficient groups were in fact obtaining copper from unrecognized sources. Under apparently similar conditions Kernkamp (1935) also obtained this result, but Hamilton et al. (1930) found that a mixture of ferric citrate and copper sulphate was a more effective prophylactic than ferric citrate alone, even when the latter was contaminated with a very small amount of copper. This variation in results is probably due to differences in the degree of restriction exercised by different workers and to the extremely small copper requirement of the young pig. Teague & Carpenter (1951) suggest that this

is about 0.05 mg. per lb. body weight daily.

Clinical signs

These have received little attention in the literature. Piglet anaemia usually affects pigs at 3 weeks of age (McGowan & Crichton, 1923) but younger pigs may also suffer. Kernkamp (1932) records clinical signs in fortnight-old piglets and death from anaemia at 8 days old (Kernkamp, 1935). Reports of such early cases should be treated with caution. At the other extreme, many affected pigs remain unthrifty long after weaning and have a predisposition to die from other causes (McGowan, 1924; Doyle et al., 1927). To an acute observer the first sign of the disease is usually an abnormal pallor. This is especially noticeable in the ears of white pigs and may be seen in the white areas of black and white animals. Affected piglets have usually grown rapidly in the first fortnight of life. Changes in the colour and nature of the faeces become apparent, although these are not well described in the literature. McGowan & Crichton (1923), Craig (1930) and Adsersen (1932) considered "diarrhoea" as a typical sign, but Graham & Thorp (1932) regarded constipation as premonitory. Luke & Gordon (1950) considered yellow or whitish dung to be a sign of anaemia and Brooksbank (1954), describing copper-deficient anaemic piglets, mentions "clay coloured diarrhoea" as a sign of this condition. At the present time piglet anaemia is frequently diagnosed on the appearance of abnormal faeces only. Piglets may now have a plump or stocky appearance (McGowan & Crichton, 1923; Kernkamp, 1932), but loss of bloom and listlessness soon occur. Jaundice may sometimes be evident (McGowan & Crichton, 1923). On castration the pigs appear relatively bloodless. They are reluctant to move, and if exercised, respiration is increased and tachycardia ("thumps") occurs. Kernkamp (1932) considered that the serous effusions in the chest could be detected by auscultation at this stage. If the pigs are not treated, sudden deaths will occur. McGowan & Crichton (1923), but not Adsersen (1932), considered this a common termination. Some pigs become cachectic, and their skin becomes wrinkled, with adherent brown waxy crusts. These pigs may die of inanition or intercurrent infections (Adsersen, 1932). Other pigs may recover. Recovery may only be partial, resulting in stunted animals which do not thrive.

Haematology

First, a summary of the haematological examinations which have been reported. The most complete observations were made by the Salt Lake City workers Lahey et al. (1952), who reported their haematological and biochemical findings, but at the conclusion of their experiments the pigs were well past weaning age and also underweight. Most workers report haemoglobin examinations, but the variety of methods employed prevents a direct comparison of results. Only McGowan & Crichton (1924); Doyle et al. (1927); Fulton (1932); Kernkamp (1932); Hamilton et al. (1933); Lahey et al. (1952); and Brooksbank (1954) recorded red cell counts whilst the characteristics of blood as seen in smears were noted by McGowan & Crichton (1924); Schofield (1930); Doyle et al. (1927); Fulton (1932);

Kernkamp (1932); and Lahey et al. (1952). Haematocrit determinations were made by Hamilton et al. (1933) and the Salt Lake City group. The white blood cell picture was briefly described by McGowan (1924) and Doyle et al. (1927) and in more detail by Fulton (1932); Kernkamp (1932); Lahey et al. (1952); and Brooksbank (1954). Platelet counts were also made by Lahey et al. (1952).

Although the above workers examined normal control animals the recorded haematological data for normal piglets are scanty. Scarborough (1930-31) reviewed the literature up to 1926, but at that time piglet anaemia was not widely recognized, and the early data for piglets are consequently unreliable. Kernkamp (1932) collected further figures and added to them the results of his own 1,100 examinations of 153 animals. He suggested "normal" ranges for red and white cells and haemoglobin in piglets reared with or without access to earth. Fraser (1938) recorded red and white cell counts of pigs of various ages and Venn (1944) and Luke (1953) also recorded white cell counts of piglets. Gardiner et al. (1953) recorded white cell counts, haemoglobin levels and haematocrits of piglets up to 14 days old. Inevitably, the numbers of animals examined have been small and it appears that the only haematocrit examinations recorded are those of Hamilton et al. (1933); Wintrobe & Shumacker (1936); Lahey et al. (1952); and Gardiner et al. (1953). It should be noted that the haematocrit readings of Hamilton et al. apparently include all the cellular elements of the blood.

Recorded observations on the red cell counts of new-born pigs (Kernkamp, 1932), the haematocrit (Hamilton et al., 1933; Wintrobe & Shumacker, 1936; Gardiner et al., 1953) and the haemoglobin (Adsersen, 1932; Schwarte, 1939; Jespersen & Olsen, 1939) show that the piglet erythron is comparable with that of adults, although the haemoglobin level and red cell counts are slightly lower. Wintrobe & Shumacker (1936) considered that the erythron of baby pigs was more mature than in any other species, excepting man. The only divergent report is that of Fraser (1938), who noted that the red cell counts of a small number of new-born pigs were well below adult figures. This generalized view of the neo-natal blood in the pig must be considered in the light of the observations of Schofield (1930) and Schwarte (1939) that there are wide variations between individuals and between litters.

There is general agreement in the literature that the blood values of pigs decline after birth. Kernkamp (1932) considered that a reduction in red cell counts and in haemoglobin continued until pigs were 7–10 days old and reductions not exceeding 30% in haemoglobin levels were normal (Kernkamp, 1935). An increase in the values began at 12 days old. Jespersen & Olsen (1939) recorded decreases in haemoglobin up to the 8th day of life. The level then fluctuated until the 15th day, after which a gradual rise occurred until adult levels were reached at weaning. Gardiner et al. (1953) recorded reduced haemoglobin and haematocrits on the 8th day, but increases had occurred by the 15th day of life. The duration of this period of decline and the actual age at which the increase commences are not

clearly defined in the literature: the variation in ages recorded reflects the frequency of the examinations reported. Day to day examinations of a large number of piglets are required to clarify the position. Kernkamp (1932) and Schwarte (1939) considered that by 3–4 weeks of age, the red cell counts and haemoglobin levels were again comparable to those of the new-born or adult pig. Hamilton et al. (1933) reported similar haematocrit findings but allowance must be made for the inclusion of all cellular elements in their interpretation of haematocrits. Jespersen & Olsen (1939) found that adult haemoglobin levels were reached only at weaning age and Fraser (1938) considered that red cell counts reached the normal adult range only after one year of life. It would appear, however, that the red blood values of 4-week-old piglets can be virtually those of adult red blood. Not all piglets achieve this potential owing to unsatisfactory nutritional and perhaps environmental circumstances (Naftalin & Howie, 1949). The recorded normal red blood values of pigs of various ages are summarized in Table I.

Compared with the figures in Table I, all the haemoglobin estimations in the literature show that the blood is hypochromic in piglet anaemia, and all the workers who have reported red cell counts have found that these were reduced. Anisocytosis, polychromasia and poikilocytosis have been reported from the examination of blood smears. Microcytosis was reported by Doyle et al. (1927); Fulton (1932); and Kernkamp (1932) from smears and by Lahey et al. (1952) from haematocrit and red cell determinations. Thus it would appear that there is a microcytic hypochromic anaemia, as in human iron deficiency anaemia. Earlier Hamilton et al. (1933) had found no decrease in the size of the red cells in piglet anaemia: this may partly be explained by their interpretation of haematocrit readings.

While the potential red blood values of young pigs are similar to those of adults, the lower normal levels are not clearly defined. Kernkamp (1935) suggested that a decrease of more than 30% from the birth level of haemoglobin constituted anaemia, but as Schofield (1930); Schwarte (1939); and Jespersen & Olsen (1939) have shown that there is a wide variation between the birth levels of litter-mates, the application of this suggestion is limited to situations where individual birth levels have been observed. Most other workers have only considered pigs to be anaemic when haemoglobin levels of 3–4 g./100 ml. or below are recorded. It is at this low level that piglets become obviously ill, indeed Baskett & Lamont (1936) considered that animals with such haemoglobin levels were likely to die, whilst Kernkamp (1932) had earlier suggested 2.5 g./100 ml. as a critical level. Schwarte (1939), however, found that pigs might have haemoglobin levels as low as 4 g./100 ml. without ill-health. Although Schwarte did not weigh his animals he considered that their growth was not noticeably affected. In view of the reported correlation between haemoglobin level and growth rate (Adsersen, 1932; Jespersen & Olsen, 1939), a reduction in growth rate would be expected, however, with such a low haemoglobin level. Schwarte (1939) postulated a wide physiological range of haemoglobin in young pigs, with the lower level apparently in the region of

TABLE I

Synopsis of the blood values of pigs as reported in the literature

R. B. C. M/cu. mm.	Haemoglobin	PCV. %.	Age of animals	Author	Remarks
6.74	80–90% (Sahli)		Adults	Scarborough (1930–31)	Average of earlier reports
5.5–7.0 3.0–4.5 5.0–6.5 5.5–7.0 2.5–4.0 2.5–4.0	9.0–13 g. 6.0– 9 g. 7.0–10 g. 9.0–12 g. 3.5– 6 g. 3.5– 6 g.		Under 24 hours 7 days 14 days 3–6 weeks 14 days 3–6 weeks	Kernkamp (1932)	Suggested normal values Access to soil from birth " " " " " Reared on concrete " " "
	8.98 g. (Newcomer)	44.1 (all cells)	Under 24 hours	Hamilton et al. (1933)	Comparable values may again be reached in 3–4 weeks
5.77 4.88			Under 3 days 1 week - 1 month	Fraser (1938)	Average of 8 animals Average of 34 animals
	10.2 g. 12.8 g. 4.8 g. (Newcomer)		New-born 3 weeks 3 weeks	Schwarte (1939)	Mean figures Grass reared Reared on concrete
	60–70% 20–30% 35–40% (Sahli)		New-born 2nd week of life " " " "	Jespersen & Olsen (1939)	Comparable figures again reached at 8 weeks Winter rearing Summer rearing
7.93	15 g.	46.3	Adult pigs	Wintrobe (1951)	
7.59	14 g.	42.0	84 days	Lahey et al. (1952)	Mean figures of 10 animals
	8.57–10.35 g. (Oxyhaemoglobin)	33.1–41.0	Under 12 hours	Gardiner et al. (1953)	Mean figures of 6 litters

4 g./100 ml. If this is the case, most piglets are sharply affected when their blood haemoglobin falls just below the lowest physiological normal. Perhaps at this level, tissue respiration is sufficiently affected to produce a train of symptoms, and at such a critical level the climate and environment might have powerful effects.

Normal variations in white cell counts make the interpretation of results difficult, but the work of Kernkamp (1932); Fraser (1938); Venn (1944); Gardiner et al. (1953); and Luke (1953) has shown that the total white cell count of normal piglets declines after birth. Usually at about 2 weeks of age the total count begins to increase; the lymphocyte becomes the predominating cell and continues to be so throughout maturity (Luke, 1953). In piglet anaemia McGowan (1924) considered lymphocytes and polymorphs to occur in approximately equal numbers. Doyle et al. (1927) suggested that total white cell counts were slightly reduced. The overall picture reported by Fulton (1932) does not seem to vary from that described above for normal piglets. Kernkamp (1932) found that non-anaemic piglets on soil had slightly higher leucocyte counts than anaemic pigs on concrete, but there was no significant difference in the differential counts. Lahey et al. (1952), using rather older pigs, found a slight neutropenia in iron deficiency, but no reduction in total counts. There was a mild leucopenia in the anaemia of copper deficiency revealed by a 50% reduction in neutrophiles and a 25% reduction in lymphocytes. The limited observations of Brooksbank (1954) on the white cell picture in copper deficiency anaemia are in agreement with those of the Salt Lake City group.

With regard to the bone marrow and to haemopoiesis generally, there are many discrepancies in the literature. McGowan (1924) reported a normoblastosis, but found that the bone marrow was aplastic. Doyle et al. (1927) considered that haematopoietic proliferation was a characteristic feature of piglet anaemia, and described haemopoiesis in the liver, spleen, bone marrow, and even in the periosteum. However, they did not consider that circulating normoblasts were especially increased. Graham & Thorp (1932) found hyperplastic bone marrow and also haemopoietic centres in the liver. They confirmed Doyle's finding of multinucleate giant cells in the spleen and described them in the liver also. It is probable that these giant cells were megakaryocytes, which are not uncommon in the liver and spleen of young pigs. Kernkamp (1932) found wide variation in the numbers of reticulocytes in pigs under 80 days old, but normoblasts were most common when the red cell count was below 4 millions per cu. mm. Fulton (1932) reported a normoblastosis in piglet anaemia and this was confirmed by Lahey et al. (1952) who also described hyperplastic bone marrow in both iron and copper deficiency anaemias. Since the reports on haemopoiesis and the leucocytic reaction of piglet anaemia are conflicting it is apparent that further studies on these aspects of the disease are necessary.

Pathology

McGowan (1924) and Doyle et al. (1927) found that the carcasses and viscera of pigs which had died of anaemia were pale and tended to be

oedematous. They found that the principal lesions occurred in the liver, heart and serous cavities of the body. McGowan (1924) described a variety of changes in the liver which he believed to be sequences in a chain of events. Many of these changes could be seen in a single liver or within one lobe of the liver, and the suggested order of occurrence was: fatty change in the central cells of the lobule, sinusoids filled with erythrocytes; centrilobular necrosis; cell spaces filled with erythrocytes, sinusoids empty; fatty change, followed by necrosis, moving towards the periphery with concurrent hypertrophy of lobule and liver. At all stages regeneration and repair were seen. The centrilobular fatty change gave the lobules white miliary centres, which became red when the necrotic cells were replaced by erythrocytes. The centrifugal destruction and replacement of hepatic cells gave rise to dark rings on the liver surface which McGowan described as "fairy rings".

Braasch (1891) had earlier noted swollen livers in the condition which he described, and the lesions reported by Doyle et al. (1927) and by Graham & Thorp (1932) are similar to those reported by McGowan. Kernkamp's (1932) description is similar also; in particular he observed that the liver might be enlarged to twice the normal size. Howie et al. (1949) suggested that unsatisfactory environment might lead to low haemoglobin and unthriftiness in pigs, despite prophylactic iron dosage. This work was extended by Naftalin & Howie (1949), who reared pigs in a cold damp byre: mortality was high and death followed coma or signs of "thumps". Although these animals had been treated with iron and their haemoglobin levels were not unduly low in early life, the lesions found resembled those of piglet anaemia, and the liver especially was characteristically affected. Naftalin & Howie (1949) considered that the liver lesions were essentially the same as those described by McGowan (1924), although in their pigs the lower edges of the liver were affected principally. Shanks (1953) described a condition characterized by enlargement of the liver and centrilobular necrosis, which he called "Big Liver Disease". Again the pathology closely resembled that found in piglet anaemia, but the disease occurred in pigs given iron supplements and in those reared on grass. Although copper deficiency has a great effect on iron metabolism, Lahey et al. (1952) reported that there were no histological abnormalities in the organs of 8 copper-deficient anaemic pigs.

McGowan did not consider that the liver lesions which he described were specific: they could be brought about by a variety of toxic agents, including organic solvents. He concluded that a major function of iron was the maintenance of healthy cells in the body and especially in the liver. Adsersen (1932) considered that the lesions in the liver and elsewhere were the result of "stasis phenomena". Naftalin & Howie (1949) suggested that the causal factor was venous congestion due to low environmental temperatures. Because of the failure of later workers (Lucas & Thompson, 1953; Lucas, 1954) to reproduce the condition described by Naftalin & Howie, the exact aetiology, like that of Shanks' "Big Liver Disease", is uncertain. In recovered, stunted animals cirrhotic or hobnail livers were commonly found (McGowan, 1924; Doyle et al., 1927), and it may be assumed that

the cirrhosis was of perilobular distribution, a form which usually follows repeated centrilobular necrosis. Elias et al. (1953) observed that the liver of the new-born pig is not lobulated with fibrous septa and they suggested that the normal liver of the adult pig is an example of subclinical portal cirrhosis. This development apparently occurs during the period when young pigs may become anaemic, and it may prove a complicating factor in the study of the liver lesions. At present it seems that these lesions may be the direct effect of iron deficiency on the liver cells or, more probably, the hepatic cellular reaction to the prevailing generalized anoxia. Until more is known about the pathology of the liver in piglet anaemia, its role in the course and symptomatology (e. g. diarrhoea) will be largely speculative.

Enlargement and dilatation of the heart were reported in cases of piglet anaemia by McGowan (1924); Doyle et al. (1927); Graham & Thorp (1932); Adsersen (1932); Kernkamp (1932); Naftalin & Howie (1949); and Shanks (1953). McGowan was struck by the enlargement of the heart, observing that it might be of such a degree as to compress the lungs into the costovertebral angle of the thorax. Heart lesions have been regarded as compensatory in nature, and Widdowson & McCance (1955) found that cardiac output is considerably increased. Surprisingly they found that enlargement of the heart was due to an increase in the number of its cells, and not to an increase in cell size as would be expected in hypertrophy.

All the authors who described the pathology of piglet anaemia reported accumulations of fluid in the body cavities and occasionally at other sites. McGowan (1924) found that the pericardium was always affected. Hydropericardium, pericarditis and haemopericardium were seen. In cases of sudden death the lungs were oedematous, and abnormal amounts of serous pleural fluid were frequently found. Serous or serofibrinous fluids and coagula were found in the peritoneal cavity. These lesions would account for the "thumping" respiration and the sudden deaths from heart failure which are a feature of the disease. The physical and chemical properties of these fluids were not described, but apparently they were regarded as fibrinous transudates of general oedema. Other lesions, such as splenic enlargement, bowel changes and renal and myocardial degeneration were not consistently reported, and are not sufficiently specific to warrant discussion.

Treatment and prevention

Although Venn et al. (1947) determined the iron requirements of piglets by analysis, and Brownlie (1955) and McDonald et al. (1955) computed iron retention from increases in haemoglobin, all other assessments of prophylactic and therapeutic measures have been somewhat empirically judged by the resultant haemoglobin levels and weight gains. Certain practical requirements, such as ease of administration and a minimum number of treatments, hamper the choice of methods. After McGowan & Crichton (1923) had shown that the condition was due to iron deficiency, later workers found that rearing on grass or the provision of soil were

effective and practical prophylactics. However, it may not always be possible to adopt these measures. Treatments that were reported as satisfactory are given in Table II.

No treatment has been found to prevent Kernkamp's (1932) post-natal "physiological anaemia". but Schofield (1930) considered that the early administration of iron prevents all but a slight reduction in blood values. In view of the peculiarities of iron absorption the reports of successful prophylaxis with a single oral dose of reduced iron are surprising. While it is unlikely that the administration of copper is necessary under normal conditions of pig husbandry, Brooksbank (1954) has shown that copper-deficiency anaemia may occur in the field. The administration of various vitamins (Doyle et al., 1927; Draper & McElroy, 1949) in piglet anaemia has not proved beneficial. Braude (1949) obtained slight increases in weaning weight by administering a liver extract, but he did not consider that these were related to haemoglobin levels. The results of Adsersen (1932); Jespersen & Olsen (1939); and Naftalin & Howie (1949) suggest that the provision of warm, dry nests for piglets is a most useful adjunct to other prophylactic methods. The importance of sunlight and exercise has been discussed under Aetiology.

Conclusions

Although the literature on piglet anaemia is considerable it is evident that present knowledge of the disease is far from complete. It is felt that the principal need is for a reassessment of diagnostic criteria, for upon these all other studies of the disease are founded. Diagnosis has been principally based on haemoglobin estimations, although the normal range of haemoglobin in the piglet is undefined, and there is considerable variation due to age, and also between individuals and between litters. Further, the M.R.C. Committee on Haemoglobin Surveys in its report (1945) specifically warns (Page 2): "In interpreting the results of any survey based on haemoglobin estimations alone, it must be remembered that the low haemoglobin may also be brought about by deficiency of a dietary constituent other than iron, or to some independent disease". In the absence of other means, diagnosis could be based on more extensive blood examinations. It is therefore essential that comprehensive observations should be made on the blood of normal piglets of various ages; the deviations from the normal could then be accurately determined. Particular attention should be paid to haematocrit examinations, which have been greatly neglected. With a reliable method of diagnosis the incidence and economic importance of the disease could be accurately measured and other causes of loss, retarded growth and diarrhoea in piglets identified and investigated. In conjunction with the observations suggested above, the bone marrow and white blood cell picture of both healthy and anaemic piglets could be examined. Additionally the possibility of increasing foetal liver iron by parenteral medication of the pregnant sow, and the occurrence and mechanism of "resistance" to the disease could be investigated.

More fundamentally, the true natures of the liver lesions, serous

TABLE II

Prophylactic treatments for piglet anaemia reported in the literature

Preparation	Dosage	Age at Administration	Authors
Ferric oxide	40 g. daily in sow's food	From birth	McGowan & Crichton (1923)
Dialysed iron Pure ferric chloride Ferric citrate and copper sulphate	5 ml. of a 5% solution 10 mg. 10 mg. of each	Daily (throughout susceptible period) " " " " " " " "	Craig (1930)
Ferric sulphate	25 mg. Fe	Daily from 1st day of life	Schofield (1930)
Ferric citrate Copper sulphate	25 mg. Fe 5 mg. Cu	Daily doses or from sow's teats	Hamilton et al. (1930)
Ferrous sulphate (commercial)	1 teaspoonful of 2.5% solution	Daily from 1 week old to weaning	Adsersen (1932)
Ferrous sulphate	Teaspoonful doses of a saturated solution (less for young pigs)	Weekly or twice weekly doses from 3–4 days old, or from sow's teats daily	Willman et al. (1932)
Copper sulphate Ferrous sulphate	1.5 g. } Sprayed on to 9.0 g. } 50 lb. soil	From birth	Moe et al. (1935)
Iron pyrophosphate Reduced iron	2 ml. solution containing 0.5 mg. Fe/ml. 500 mg.	3–6 daily doses during 2nd week of life Single dose on 7th day of life	Foot & Thompson (1938) Foot & Thompson (1949)
Reduced iron	300 mg.	Single dose, 6–8th day of life	Schofield & Lloyd Jones (1939)
Reduced iron	210 mg.	Weekly doses beginning before 4th day of life	Draper & McElroy (1949)
Iron dextran complex	100 mg.	Single injection 7th day of life	Barber et al. (1955a)

effusions and diarrhoea characteristic of the disease require elucidation, together with various facets of iron metabolism. In particular it is not known whether the increased incidence and severity of the disease in the colder months is a direct effect of environment on haemoglobin synthesis or is due to the greater requirement and possibly reduced absorption of iron by pigs subjected to low temperatures.

SUMMARY

The literature on piglet anaemia is reviewed and discussed. This is an important disease of young pigs, principally caused by iron deficiency occurring under intensive systems of swine husbandry. Close attention must be paid to prophylactic measures in order to avoid severe economic loss from death, disease and reduced growth rate, especially during the winter months. The disease is clinically characterized by pallor, increased respirations, diarrhoea and death. The main lesions are lowered haemoglobin, reduced red cell counts, liver dystrophy, cardiac enlargement and serous effusions. Attention is drawn to certain aspects of the disease which are as yet incompletely understood.

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Rinderpest: Some Notes on Control by Modified Virus Vaccines. I

BY

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INTRODUCTION

According to Gamgee (1866) (Henning, 1949), rinderpest or cattle plague has been recognized as a disease entity from the earliest times. The most recent outbreaks in Europe occurred in Belgium and France in 1920 and in Malta in 1946-47 (Gatt Rutter, 1949). An outbreak also occurred in the Rome Zoological Gardens in 1949 (Tassi, 1949) and Boldrini (1954) recorded a case in an African buffalo landed at Trieste, episodes which illustrate that re-introduction of the disease is possible even now, although extension from such isolated incidents is unlikely in any but the most abnormal circumstances. The disease remains endemic in many parts of Asia, Africa and the Far East, where its control constitutes almost the major problem of the veterinary services.

From time to time assertions have been made that eradication was within sight. Ultimately this must prove correct, but it is becoming increasingly obvious that there is no single "touchstone" and in many less fortunate territories, the requisite conditions do not exist yet; among the essentials are a stable administration, an efficient veterinary service and access to a laboratory for the centralized production and storage of vaccines.

In East Africa, rinderpest has been successfully contained for the last two decades, but it has always been present; the disease has sometimes appeared inexplicably in the centre of areas free for many years and from time to time the virus has been introduced from foci which are always present outside the borders. In any country the problem is one of economics and in those with slender resources rinderpest control certainly retards progress in other spheres of animal industry. The part played by game has been the subject of bitter controversy but it is well to remember that many countries with a profusion of fauna capable of maintaining the virus, have eradicated the disease and it seems likely that the National Parks movement, in Africa at any rate, will resolve the problem. Nevertheless, it is unfortunate that the recommendations of the Rinderpest Conference of 1948, for the investigation of the disease in game, have never been implemented, for it is an aspect of research long overdue and which might prove fruitful.

The introduction of methods of artificial immunization high-lighted

the problems of variation in resistance of the races of cattle and even of individuals within breeds; the search for more suitable vaccines was stimulated and there is now a formidable literature on the subject, some of which is very contradictory. It is understandable that the properties of the virus, its variants, the serology and many other aspects were neglected until recently, because of the need to control the disease, but now the progress of eradication may largely depend upon the resolution of the confusion which exists about the fundamental properties of the various vaccines.

METHODS OF IMMUNIZATION

The evolution of rinderpest prophylaxis has an interesting history but as most of the early references have been cited by Henning (1949) and as Mornet (1954) has recently traced the sequence to the present day, the various methods are described very briefly here.

Unterberger (quoted by Scott, 1955) as early as 1744 and Dobson in 1754, attempted to protect animals with the secretions and excretions from natural cases. The virulence of the infective agent defeated these attempts, but Koch used bile from infected animals with success in 1897 and was able to show that bile neutralized the virus. According to Kleine (1941) the possibilities of using material passaged in goats were foreseen by Koch at this time, but were never put into effect.

The serum from recovered animals was shown to protect by Semner (1893) and the method was explored by many workers; Pool & Doyle (1922) described their investigations in detail, and for many years serum alone was the only means of controlling the disease in India.

Kolle & Turner (1898) first described control of the virus reaction with immune serum and from 1910 the "serum-simultaneous" vaccination of cattle was carried out in East Africa, when some measure of control of the epizootic nature of the disease was established.

The report by Kakizaki (1918) of a glycerol-inactivated tissue vaccine led to an improved phase with a method which did not create new centres of infection in the field and variations are still in use to-day, although present trends favour the adjuvant type of vaccine.

It is generally accepted that adaptation of the virus and evolution of experimentally induced variants in goats and rabbits, began with the work of Edwards (1927) although previous workers had already reported some degree of attenuation after goat passage and had used goat blood as a source of virus. The development of freeze-drying techniques has revolutionized the control of rinderpest by the modified virus vaccines which should have some application in most countries, however specialized their requirements.

The inactivated tissue vaccines and the attenuated variants continue to satisfy modern requirements; passive protection by serum still has a limited application under special circumstances but no longer has any place

in general rinderpest control. With both methods, it is as yet undetermined when immunity is best reinforced, particularly with those attenuated viruses which give a short protection. Similarly little is known of the effect of vaccines upon resistant animals and young animals, so that there is some risk in the movement of vaccinated stock, especially into countries where the cattle population is highly susceptible.

Inactivated tissue vaccines

Simple vaccines of this type, that is without adjuvants, have served a useful purpose in earlier campaigns against rinderpest; their disadvantages are well known and the gradual decline in immunity involves the possibility of the occurrence of inapparent infection with field virus. There is no real problem with revaccination but there is also no serological evidence of a secondary response to the antigen in the face of a waning immunity. Killed vaccines have been improved by the incorporation of adjuvants, alone and with acid-fast organisms. Their inherent disadvantages remain however and those to which killed mycobacteria have been added, although giving a higher grade and longer duration of immunity, have in their present form an unfortunate tendency to provoke unsightly reactions, and to damage the tissues, so that parts of the carcass of slaughtered animals are unacceptable.

Modified viruses

All attenuated viruses according to Cox (1953, 1954) are of two types, and they provoke a more durable immunity than killed vaccines. They are (a) those attenuated by passage in an unusual host, such as rabies, yellow fever and distemper and (b) variants of low pathogenicity which have been found in the natural host, for example certain strains of Newcastle disease virus and vaccinia. Those in the former category, including rinderpest, are said to be "fixed", since their passage in the natural host does not elevate the virulence to that of the natural disease.

With the range of modified rinderpest viruses which can be produced at relatively low cost, it seems clear that they will prove the mainstay of control. Not all workers are agreed upon their properties, however, and because of this they are still regarded with reserve or used in circumstances in which their efficacy becomes open to criticism. Crawford (1947) has drawn attention to one aspect of goat virus, which when introduced into a new territory, often causes very severe reactions with considerable mortality. There are some grounds for care therefore and several passages of any attenuated virus in the host animal which is available locally, may be necessary before the properties can be assessed in a new environment.

THE MODIFIED VIRUS VARIANTS OF RINDERPEST

I. Goat-Adapted Virus

History

Beginning with Koch (1897) several workers have reported some attenuation of the rinderpest virus after a variable number of passages in goats (Schein, 1926; Topacio, 1926) but since these were never followed up, their importance was clearly not recognized at that time. The credit for the development and practical application of this finding must remain with Edwards and his co-workers at Mukteswar in India. Starting in 1922 their researches began a revolution in rinderpest control. Edwards (1949) has written an account in retrospect and gives the earlier references to attenuation. He began with direct and alternate passages in goats after a rabbit-adapted virus which he had maintained for over a year was lost. The immediate aim was to produce a virus free from pathogenic protozoa, suitable for the "serum–simultaneous" vaccination of cattle but that the Mukteswar goat virus had become attenuated became apparent when three cattle used as controls, survived after showing a mild reaction typical of rinderpest. Edwards (1930) attributed this observation to his colleague Menon, but confirmed the attenuation in the highly susceptible Himalayan hill cattle. Other similar observations were soon forthcoming and although several records were made independently in various Government of India reports, the earliest references in the literature are those of Stirling (1932, 1933). A report was made by Saunders & Ayyar (1936) of 150 serial passages of the virus in goats and the results checked in cattle showed that attenuation began at the 80th passage. In Burma, Pfaff (1938, 1940) was the pioneer of dried goat virus vaccine and recorded what has since come to be known as the "interference phenomenon". The important African experiences began with the work of Daubney & Hudson in 1936 and have been reported by Daubney (1937, 1948). The method has taken a long time to become recognized outside India and E. Africa considering that it has proved such a potent and successful weapon in the hands of those cited; it is now more popular and Mornet (1948) has reviewed the advantages of goat virus when compared with other vaccines.

Susceptibility and resistance to goat virus

When considering this subject attention must be confined to the "fixed" goat virus and not to the bovine virus in process of adaptation, for during that process the virus becomes progressively more virulent for the goat and less virulent for cattle.

There are numerous references in the literature which seem to indicate that goats vary in susceptibility. In Nigeria, goats from the Kano district have been reported to be more easily infected than those from other areas (Henderson, 1945). Highly susceptible goats were reported in India by Sharma & Ram (1955) while on the other hand Girard & Charitat (1947) considered certain breeds in the Sudan to be resistant and Saquet & Tro-

quereau (1951–52) reported that it was necessary to inoculate ten animals in order to obtain one good reaction. Daubney (1948) believed that there was variation from goat to goat, taking the titre of virus in individual spleens as the criterion and that habitat, breed and age all influenced the reaction. It seems clear that goats do differ in their resistance to the virus, but no evidence has been produced to show whether this resistance is due to previous experience with the virus or whether it is inherent in some breeds.

Variation in cattle has been reported extensively and susceptibility to goat virus appears to be related to the severity of the natural disease in the races and breeds. The zebu (Bos indicus), especially the plains cattle of India, are the least susceptible while European (Bos taurus) breeds are most susceptible, with the crosses of the two somewhere in between. In some countries hyper-susceptible cattle are to be found, Himalayan hill cattle in India, Ankole, Benghazi and West African Shorthorn in Africa, while Japanese black cattle appear to be the most highly susceptible of all. Daubney (1937) considered that the Mukteswar strain of goat virus was too severe for use in Kenya zebus, but there have been no comparisons of the various strains and sub-strains of goat virus which exist to-day. Animals below six months of age were said to be resistant by Bachan Singh (1939) and the fact that calves do not appear to be suitable for vaccination is now well recognized. Cattle which show complete resistance to the virus are usually considered to have acquired an active immunity.

Buffaloes show a variation in susceptibility which appears to be related to the geographical distribution of the species. In the Far East the buffalo is very susceptible; in India, variable; and in Egypt, very resistant (Pfaff, 1940; Hudson & Wongsongsarn, 1950; Daubney, 1949).

Certain breeds of sheep are very susceptible in India according to Sharma & Ram (1955).

Pigs have been shown to be susceptible to the virus by Hudson & Wongsongsarn but these were the indigenous pigs of Siam and there are no reports of infection of European breeds of pigs.

Various species of game have been shown to be susceptible as judged by reactions to injections of goat-adapted virus and subsequent challenge with bovine virus; blood from such animals was also shown to cause a mild reaction and to protect cattle (Reports of the Veterinary Department Kenya 1936 to 1942).

Virulence of the goat-adapted virus

Passage of the goat-adapted virus through cattle did not cause any increase in virulence according to Waddington (1945), and Brotherston (1951b) reported that three alternate passages in goats and cattle did not alter the character of the reaction for either animal. However, Hudson (1950) has stated that on repeated passage in goats, the attacking power

for cattle becomes reduced and that one passage in the ox will restore it. This "out-passage" of an alleged fixed virus, also advocated by Edwards (1949), would appear to be unnecessary if stocks of freeze-dried material are kept, but the whole question merits further research for it implies that the virus is capable of an alteration in character after a brief change of environment.

After passage of the virus in the Siamese pigs, Hudson & Wongsongsarn reported that the virulence was reduced for buffaloes. After three serial passages in the warthog no increase in the temperature reaction was noted and the Report of the Veterinary Department Kenya for 1942 states there is no evidence that Kabete attenuated goat virus (KAG) possesses for any game species a virulence any greater than it has for cattle; the species examined were buffalo, giraffe, oryx, bushbuck, duiker, and warthog; one reedbuck died after inoculation with the virus.

Route of infection and transmission of goat-adapted virus

Goat virus is usually injected subcutaneously but other routes have been used. Daubney & Hudson (1940) attempted to intensify the pneumotropic character exhibited by earlier passages in goats, but 50 serial passages intranasally showed no increase in this effect. Scarification was successful in sheep and goats (Sharma & Ram, 1955) and in cattle (Siddiqi, 1944) while Scott (1952) has described vaccination of cattle by the intradermal route. Drenching with secretions and excretions from reacting cattle has been described, with success (Cornell & Oonywongse, 1941; Mitchell & Le Roux, 1946).

Contact infection from reacting animals has never been established. Brotherston (1951) described one instance when a control ox developed a typical reaction, with subsequent immunity, through contact with a fellow ox reacting to the virus, which had a broken and bleeding horn, but this was an extreme case for infected blood might have been transmitted by a variety of means. Contact experiments have been tried in Kenya with game, but the few trials were negative.

Temperature reaction to the goat-adapted virus

Figures in the literature show a marked variation in the thermal reaction of goats, and most workers regard a good thermal reaction as essential for a high yield of virus. In Kenya, goats were assessed upon the pattern of the individual temperature rise and not upon a particular increase, over the period of four days following injection with a large dose of virus. Cilli (1951) described several degrees of thermal reaction in goats in Eritrea and reported that virus could be recovered from the spleen of non-reactors.

Susceptible cattle often have sustained high temperatures; Daubney (1949) stated that 95% of zebu cattle in Kenya reacted and that 100%

were thereafter immune. Hudson (1950) suggested that although some few cattle might become immune without showing this characteristic temperature, any proportion over 10% in this category should be revaccinated. The febrile reaction appears between four and five days after the injection of virus but personal experience suggests that the time of maximum temperature depends upon the resistance of the animal and the concentration of the virus; low concentrations delay the response. The amount of virus injected does not influence the appearance of the temperature reaction (Pfaff, 1940).

Buffaloes, sheep, pigs in Siam, and game have all been reported to react thermally in varying degree which lends support to the contention that this property is an index of susceptibility to the natural virus.

Clinical symptoms other than temperature

Edwards (1927) and Stirling (1933) have described the syndrome, and the latter mentioned four stages, incubative, febrile, pneumonic and the stage of diarrhoea but these were recorded at comparatively early stages of adaptation. The Kenya Veterinary Department Report for 1936 also mentions broncho-pneumonia and pleurisy but these signs disappeared on passage and could not be induced later by intranasal instillation. Cilli (1951) described atypical and typical syndromes of various degrees. In Kenya, the clinical signs seen in goats during the four days of infection were no more than those usually associated with a rise in temperature.

During the early work on attenuation in India and Africa, the symptoms in cattle were described in detail. Since that time there has been no description of note with regard to the different types of cattle although the reaction varies according to the susceptibility. Most workers agree that cattle experience considerable stress after vaccination and that bad climatic conditions exacerbate the symptoms. The milk yield is affected even in zebus according to Bachan Singh (1939) while the stress may allow such intercurrent diseases as coccidiosis and trypanosomiasis to flare up. Diarrhoea is usually a sign that the animals are undergoing a severe reaction to goat virus.

Pathology and histopathology

This aspect has also been neglected since the first reports were published. In goats pneumonic and gastro-intestinal lesions have been described but there is some doubt as to whether the lung lesions are due to the virus or to an intercurrent disease, such as pleuro-pneumonia, which is very common in these animals. Cilli (1951) described the histopathology in the goat.

Mortality

In goats the mortality has been reported as increasing after the initial adaptation although some workers have described an immediate and constant high death rate and others a consistently low rate. More recent

publications show a similar discrepancy, Daubney (1949) mentioned 90% and Saquet & Troquereau (1951–52) up to 50%; this may be due to variations in resistance, in different countries. The severe effect with high mortality in India has complicated the problem of immunizing certain breeds of goats against the natural disease, although it is difficult to appreciate the rationale of using goat virus to immunize goats.

In cattle the mortality rate varies with race, breed and resistance of the individual, so that as a measure of the stage of attenuation or virulence of the virus, results can only be interpreted as applying to particular cattle. The death rate in cattle should be regarded more properly as an index of the resistance of the type of cattle. D'Costa (1938) quotes 70% for Himalayan hill bulls; the rate for zebu cattle varies from less than one per thousand (Pfaff, 1938) to 5% when animals are in poor condition. Of cattle of European type, 7% may die. The Mukteswar strain of virus killed 18% of Kenya zebus according to Daubney (1949) while the Egyptian substrain of the Kenya virus caused the low rate of two per thousand in local cattle which were not of the zebu type. Animals reacting severely may die from secondary causes and low grade intercurrent disease, such as trypanosomiasis, may preclude the use of the vaccine even in the more resistant types of cattle in some parts of Uganda and the Sudan; this factor probably accounts for the popularity of the milder lapinized virus, although this was never intended for use in essentially resistant breeds. A mortality of 50% has been recorded in buffaloes in Siam by Cornell & Oonywongse (1941).

The virus in the tissues

As there is a viraemia in reacting animals, one would expect the virus to be demonstrated in all tissues with a good blood supply. Blood, spleen, gastric mucosa, intestinal mucosa, nasal secretions and faeces have all been reported to contain virus by early workers but the titre of the virus from tissues has never been adequately investigated in goats as the experimental animals. Virus in low concentration causes a delayed thermal reaction and it is difficult to assess an endpoint in the absence of pathognomonic lesions in the animals which die before their immunity can be determined.

The titre of goat virus in cattle was reported by Edwards (1927) who demonstrated virus at a 1:10,000 dilution per ml. in blood; the Report of the Veterinary Department Kenya for 1937 mentions 60,000 minimal infective doses per g. of spleen as the highest titration of fresh material. Titrations of both fresh and dried material are likely to vary so greatly in technique and in the animals used that it is doubtful if they mean very much except to those actually engaged in the production of tested vaccines. Certainly sufficient attention has not been paid in the past to the inherent errors of virus titrations and especially in respect of the numbers of animals used for the test.

Cornell & Oonywongse (1941) working with buffaloes and cattle showed

the virus to be present in the blood between the fifth and fifteenth day following the injection and Pfaff (1940) using up to four animals to each dilution showed that dried spleen at 1:40,000 would immunize buffaloes. Nasal secretions of reacting animals have been proved to contain virus (Waddington, 1945) but the distribution of the virus has not been systematically measured or investigated.

Resistance and viability of the virus

Few detailed experiments have been published on the effects of physical and chemical agents — apart from cold, heat and desiccation — on survival. Sharma & Ram (1953) indicated that the virus resisted glycerol for 96 hours at 32° F. Fresh blood was kept in cold store for 19 days and spleen for 40 days and both were proved to contain viable virus by Phadke et al. (1937). Dried virus under vacuum was stored for six weeks at −4° to −22° F. and then survived a further 39 days at 66° to 73° F., while a serum saline extracted product retained potency for four days under vacuum at 98.6° F. (Brotherston et al., 1951). In the same series of experiments in Kenya, the virus reconstituted in normal saline at pH 7.4 survived for 56 hours at 72° to 80° F. Dried virus sealed in glass ampoules, with a moisture content of 1–2%, did not appear to have lost titre after eleven and a half months' storage at −4° to −22° F. (Purchase et al., 1953).

Immunity

Natural rinderpest in sheep and goats has never been recorded in Africa, although herds were alleged to have been infected in the Orange Free State and in Koch's view, the animals might show no initial infection and yet be capable of transmitting the virus. Goats do become infected in other parts of the world, however, and the literature has been cited by Scott (1955). In India vaccination of goats and sheep is practised; Bawa & Haji (1939) used goat virus to control an outbreak in sheep, and Lall (1947) employed goat virus with serum in goats and found the immunity to be complete. Sharma & Ram (1955) reported that the reactions to either intradermal or subcutaneous injection of the virus were equally unsatisfactory because of their severity in some breeds of goats.

Cattle which show a thermal reaction to injection of goat virus are invariably immune thereafter and Hudson (1950) maintains that a response of 90% of the individuals is necessary to achieve a satisfactory level of immunity in a herd. Immunity has been reported without an accompanying reaction and the virus has been recovered from the blood (Cornell & Oonywongse, 1941) while Edwards (1949) discussed immunity following upon degrees of thermal response. Immune serum used with the virus may prevent a reaction and subsequent immunity (Banerji, 1933; Daubney, 1949). The question of the best age at which animals should be vaccinated and the duration of immunity in calves from immune dams has not been agreed; Bachan Singh (1939) considered animals over six months suitable while Marcque & Falley (1950) reported calves suckled by immune dams to be resistant. Serological evidence of an immunity transferred by the colostrum

has not been proven.

Buffaloes have been reported as too susceptible for immunization by goat virus (Joshy, 1935) but other workers have successfully immunized them with and without serum. The variation of susceptibility of different breeds probably acocunts for the conflicting reports.

An outbreak in camels was stopped by the injection of goat virus so these animals presumably can be immunized also (Srinivasan, 1940). Experiments with game in Kenya have proved that certain species can be immunized.

Duration of immunity has been reported as up to five years and there is no evidence to show that animals, once vaccinated with goat virus, have become susceptible again. The duration is certainly almost life-long as far as cattle are concerned, but one feels that sufficient attention has not been paid to the possibility of reinforcement of immunity through contact with the natural disease in the field.

Pfaff (1938) first reported that the inoculation of the virus 48 hours in advance of virulent bovine virus, prevented infection with the latter. Such an "interference phenomenon" has since been described between other viruses.

Serology and antigenicity

The serology has been neglected because of the lack of a suitable *in vitro* test. The expense of neutralization or protective tests in cattle is prohibitive and the goat does not give clear-cut results with low concentrations of virus. Circulating antibodies are produced and French (1936) described the antibody as being attached over a wider range of the serum globulins in goats than in cattle, but this did not refer to a fully goat-adapted virus. Pellegrini & Guarini (1952) demonstrated complement-fixing antibodies in vaccinated cattle and Brotherston (1955) described preliminary experiments with a conglutinating complement absorption test. The relationships between different strains of goat virus and the parent bovine virus have not been described. Daubney (1949) showed that the Mukteswar goat virus protected cattle against challenge with a Kenya bovine virus and the Kabete attenuated goat virus and its substrains has been used all over Africa with success. The pleurality of rinderpest virus has never been seriously suggested and the examination of antigens of bovine, caprinized and lapinized virus-containing tissues showed them to be very closely related serologically (Brotherston, 1955). However, Edwards (1949) working with British cattle in England suggested that the Indian and African goat viruses were not completely similar antigenically and that the adaptation of rinderpest to the goat not only reduced the pathogenicity for the ox, but also modified the immunogenic components. The concept is a startling one but not unreasonable if one subscribes to the modification of goat virus by "outpassage" in cattle.

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Bloat

BY

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Over the past four years there has been a considerable increase in research into the problem of bloat. This general review will deal mainly with these recent contributions and the light they shed on the aetiology and treatment of the condition. Excellent reviews of earlier work have been contributed by Cole et al. (1945) and by Dougherty (1953).

DEFINITION AND TYPES OF BLOAT

Tympanites or bloat is a disorder of ruminants which occurs in all parts of the world where grazing of legumes is practised. It is the result of interference with elimination of the gas which is normally produced by the microbial fermentation of food in the rumen and reticulum. There are two routes by which gas is eliminated from the forestomachs, (a) by gas diffusion between rumen and blood, and (b) by eructation. Though the former may make a considerable contribution to the clearing of gas from the rumen particularly in regard to CO_2, the latter is the route of major importance and it is with this pathway that we will be principally concerned.

Bloat is a state which has been classified as "chronic", "subacute" or "acute". As Cole et al. (1945) have stated acute bloat depends on the nature of the food, whereas chronic bloat is due to an abnormality in the state of the animal. These terms are not altogether satisfactory as foods such as grain and hay can lead to subacute bloat of more or less chronic nature (Smith et al., 1953). Further, subacute bloat may be only an early stage of the acute condition. On the other hand, in the early stages some factor such as foaming of the rumen contents could cause the initial retention of gas. Later when a critical pressure is reached this pressure may interfere with the eructation reflex and cause the more distressing form of the condition. Acute bloat is classified as either (1) frothy bloat, or (2) free gas bloat according to whether there is foam or free gas present in the rumen.

In this review we will be dealing mainly with the acute type that is usually associated with the grazing of legumes.

EXTENT OF LOSSES DUE TO BLOAT

Mortality

It is very difficult to obtain an accurate estimate of the seriousness of the bloat problem. The number of deaths occurring each year is only a portion of the loss encountered by the dairy farmer.

Surveys on the incidence of bloat and deaths from it have been made in a number of different parts of the world. Nichols (1954b) reports that

a survey of over 8,000 farms suggested that about 30,000 cows develop bloat in Wisconsin each year. Of this number nearly 6,000 died, representing an annual loss of over $2,000,000 in Wisconsin alone.

Barrentine (see Marston, 1953), quoting survey figures in the Mississippi Basin area, gives 6,000 cases in 1949 with 600 deaths, and in 1950, 8,000 bloat cases were reported with 1,000 deaths. It has been estimated in the U. S. that the disorder results in production losses to the cattle and sheep industries of approximately $40,000,000 annually.

Menzies (1953) reporting on a bloat survey carried out in England and Wales found that the proportion of grazing animals which developed bloat varied between 2 and 16% and the mortality rate amongst the affected cattle was generally 15–50% but in rare cases reached 100%. Lawrence (1954) states that fortunately in England bloat does not reach very great proportions nationally. Nevertheless, the losses sustained can be extremely serious. It is alleged that trouble from bloat has increased in recent years and that the modern grassland system based on intensively managed leys is partly to blame.

An extensive survey was carried out by the New Zealand Dairy Board in the 1953–54 seasons. It showed that more than one dairy cow in every 200 died from bloat in that season in New Zealand, which represents a loss of 10,000 cows. Of the 4,943 farmers who provided information just over 12% considered it to be a serious problem in their herds in the 1953–54 season. In deciding whether or not bloat was a serious problem in their herds, farmers were influenced by such factors as the length of time over which bloat was occurring in their herds, the number of cows affected and the worry and inconvenience which resulted from their efforts at control, as well as the number of cows actually lost. The farmers who considered bloat to be a serious problem lost 2.65% of their cows from this cause compared with the Dominion average of 0.62%.

Loss of production

The number of cows dying from bloat is by no means a measure of the full economic loss. It takes no account of the loss in milk production both from animals which die and those that recover. Besides the loss of production during bloating, lactation after recovery is often curtailed, or may be terminated.

The loss in production is chiefly due to the decrease in food intake. Even mild bloat will cause a reduction in food intake with a subsequent loss in milk production. This was illustrated in the average dry matter intake recorded for the various degrees of bloat in stall-fed identical twins (Johns, 1954).

A further illustration of the effect of bloat on intake and the effect of oils in counteracting this effect was illustrated in experiments by Reid & Johns (1956). A record of girth increase in bloating animals was made using the "Tympanograph" designed by Reid (1956a) and the intake of herbage measured.

There was a girth increase on normal feeding on clover due to rumen filling with an intake of approximately 8lb. dry matter. When the animal bloated a considerable increase in girth occurred and a reduction of 50% in dry matter intake resulted.

When the animal was rapidly becoming bloated and had ceased eating it was drenched with oil. This caused a decrease in girth measurement to a value considerably below that of the normally fed animal. The animal also started to eat again. The predrenching of oil resulted in practically no change in girth measurement even though the animal ate approximately the same amount of clover as on non-bloating material. The food was definitely bloating clover as shown by the reaction of the twin animal.

Miscellaneous

Outbreaks of severe bloat can cause havoc with the normal schedule of farm operations. It may prevent a farmer carrying out his normal duties, upset his grazing rotation, and force him to use up reserves of hay and silage. The physical and mental strain on a farmer and his family has to be experienced to be appreciated. It may make the farmer hesitate to use clovers in his seeds mixture.

FACTORS AFFECTING THE OCCURRENCE OF BLOAT

Food

Bloat is most commonly associated with the grazing of lush green legumes. Lucerne, white and red clover, ladino clover, sweet clover and peas have all been incriminated at different times. The major cause of bloat in the United Kingdom and New Zealand is probably white clover (N.Z. Dairy Board Annual Report, 1954) while lucerne is of considerable importance in South Africa and in the United States, particularly in irrigated areas. Bloat is frequently experienced in New Zealand on chou moellier and has been occasionally reported on green cereals, lush young grass, turnips and cabbage. Bloat on lush young spring grass appears to be not uncommon in England and has been produced experimentally on rapidly growing young ryegrass in New Zealand (Reid, private communication).

Little work has been done in comparing the bloating potential of more recently bred strains of clovers with older strains. The former are in general more vigorous and consequently may require more careful management to prevent their becoming too dominant a species in the pasture association.

Troughton (1955) compared 3 strains of white clover in association with perennial ryegrass over 3 years for their ability to produce bloat. Only slight differences were observed between the 3 clover strains. The S.100 mixture produced the most bloat, followed closely by S.100 Nomark which produced slightly more than S.184. Comparing the herbage yields of the mixtures within any one year, in particular 1949 and 1950, the amount of bloat occurring on a mixture varied directly with the total amount of herbage offered and with the percentage of white clover in the herbage. On the other hand, the amount of bloat in different years does not appear to correspond with either the quantity or the composition of the herbage offered to the animals in those years, since in the third year the yields were as high as, or slightly higher than, in the first harvest year, but the frequency of bloating was markedly lower. Troughton in listing the factors that caused a decrease in bloat over the 3 years considered that the only one which might have caused the yearly reduction in the amount of bloat

was the age of the sward.

Both fertilizer treatment and soil mineral deficiency have been claimed to induce bloat. It is difficult to determine, however, whether the effect of treatment has influenced the chemical composition of the plant or whether, by making conditions more suitable for growth, it has altered the balance of species in the pasture association.

Henrici (1952) found in grazing experiments on lucerne in the Veld Reserve, S. Africa, that not a single case of bloat resulted, whereas under similar meteorological conditions in another district bloating was observed. Henrici states that saponin was present in the lucerne from the bloat district and absent from that of the Veld Reserve. She believes that the difference may be due to a soil factor. Troughton (1955) found that the quantities of soluble P_2O_5 and K_2O in the soil varied almost inversely with the amount of bloat, the blocks producing most bloat being poor in P_2O_5 and K_2O. Barrentine (see Marston, 1953), presenting preliminary findings, believed that the results of his fertilizer trials indicated that very fertile or highly fertilized soil produces clover with a higher content of some bloat-producing substance than do less fertile soils. Shawyer et al. (1954) obtained increased bloat incidence on ladino clover heavily dressed with superphosphate and potash.

There appears to be no doubt that bloat incidence varies from district to district irrespective of soil type and fertility.

Climatic

The correlation between bloat and weather conditions appears to be very marked in some districts. However, the theories as to the type of weather involved are many and varied, varying from "wet weather", "clear dry weather", "dull humid weather", "dew" and "frost" (M'Candlish & O'Brien, 1933; Cole et al., 1945) or "drought" (McIntosh, 1937). In the controlled experiments of Newbold (1953) and Johns (1954) none of these conditions could be consistently blamed for the production of bloating red clover.

Troughton (1955) found no correlation between maximum and minimum temperatures, rainfall, sunshine or humidity and the quantity of bloat produced per day. He did find that the amount of bloat decreased with the increasing altitude of his experimental blocks. Alder (personal communication) found bloat in wet and dry conditions with no correlation with weather conditions. It appears likely that the climatic and soil factors necessary for the development of the bloating potential in clover will be determined only when the substances present in the plant responsible for bloat production can be estimated.

Individual susceptibility

Bloat appears to be more common in cattle than in sheep when grazing mixed pastures in the United Kingdom, U. S. A. and New Zealand. Fatalities in sheep grazing lucerne appear to be more common in South Africa than in most other countries.

One of the puzzling features of bloat is the variation in susceptibility among individual animals. It is common experience that some cows within a herd become repeatedly affected while others never show any sign of bloat.

In general it appears that approximately one-third will bloat fairly frequently, one-third rarely, and one-third not at all. However, the difference between animals seems to be only one of degree, because if clover is potent enough all animals in a herd have been known to bloat (Cole, personal communication).

There is strong evidence that the tendency to bloat is an inherited characteristic (Knapp et al., 1943). Hancock (1954), in grazing experiments with ten sets of identical twin cows, found that twin members of the same set showed a remarkable similarity in bloat behaviour and that there were at the same time very great differences between sets. As these twins were a random sample of dairy cattle in so far as bloat susceptibility was concerned, it was concluded that these between-set differences were mainly genetically determined. Hancock noted that 60% of the cows selected were the daughters of one bull, although only 30% of the herd were his progeny. This illustrates how easily the number of bloat-susceptible cows could be increased by the use of such a bull in an artificial insemination scheme.

Johns (1954), using some of Hancock's pairs of twins in stall feeding experiments, found a greater difference in bloating behaviour between pairs of twins than between members of either twin set. It was calculated on the basis of bloat experiments that one pair of twins could replace nine ordinary cows in bloat trials. These figures must be treated with caution, however, as they are based on only two sets of twins. It is obvious that the use of identical twins in controlled bloat experiments has a decided advantage over a random selection of animals.

The basis of the hereditary differences between animals would appear to be due to the state of the animal or the condition of the rumen contents rather than to anatomical differences, as the susceptibility of the pairs of twins does not necessarily remain constant. During a feeding programme lasting several months while moderate bloat is being experienced some pairs will be susceptible for a period and others not. Later the non-susceptible group may become susceptible and vice versa but even here the members of a pair behave similarly.

Huffman (personal communication) has noted that in the production of "feed lot" bloat there is a greater similarity between members of a set of twins than between sets in their time of adaptation to the susceptible condition. Clark (1950b) found that by passing CO_2 through a fistula into the rumen of animals on lucerne an occasional animal would tend to retain the gas which could be released by an antifoaming agent. The sudden appearance of a "bloater" could not be explained. It appears, however, that the condition of the rumen ingesta at the time of feeding bloat-provoking herbage is as important as the nature of the herbage itself.

It has long been believed that the difference between animals in their susceptibility to bloat is due to variation in their total intake or rate of intake of herbage and that this accounts for the differences between milking cows and dry cows or beef cattle. However, it appears from the work of Hancock (1954) and Johns (1954) that there is no relationship between rate of food intake or total intake and bloat severity.

Weiss (1953b) pointed out that the psychological condition of the animal may play an important part in the pathogenesis of bloat, he saw bloat occurring in a herd coincidental with the change over from hand to machine

milking. Reid (personal communication) experienced cases where a herd of cows, which had experienced bloat in a particular field, bloated while being driven again to that area. It appears that nervousness may be a factor in proneness to bloat.

Experimental production of bloat

Legume bloat As Weiss (1953a) stated: "The greatest single factor responsible for the failure to elucidate the pathogenesis of bloat has been the difficulty encountered in reproducing the natural condition." He summarized the observations of Cole et al. (1945) on certain essential conditions necessary before bloat can be expected to occur. Though these are perhaps the optimum conditions it does not appear from the author's experience that bloat will always occur even though they are fulfilled and bloat does sometimes occur when they are not. It was found by Newbold (1953) and Johns (1954) that bloat could be produced in stall-fed animals fed red clover, with sufficient regularity for experimental purposes. The conditions necessary for bloat were not defined except that soil moisture occasionally seemed to be the limiting factor. However, even when this was rectified by irrigation rapid growth did not necessarily produce bloating fodder. Barrentine et al. (1954) in grazing experiments with steers on ladino clover found that neither the moisture content nor lushness of the clover appeared to be a factor in the incidence or severity of bloat.

A number of workers have been able to produce bloat by drenching with juice expressed from legumes (Parsons et al., 1952; Lindahl et al., 1954; Newbold, 1954; Ferguson & Terry, 1955). Several of these workers found the sheep to be a useful test animal as long as the animal had been grazing on legumes and not on grass. However, it is imperative that proper controls should be run in this type of experiment. Ferguson and Terry found that slight bloat was produced by drenching 300g. of sugar. They state: "Although the administration of quercetin and/or sugar caused some bloat, it is thought that water alone or the excitement of drenching might have produced the same effect." Lindahl et al. (1954) also produced bloat in sheep and goats by dosing with rather large amounts of lucerne saponin. Here again it was essential to have the animals grazing legumes to obtain a positive reaction. The use of other commercially available saponins gave negative results.

The use of the sheep as a test animal is of considerable importance as it reduces the amount of material necessary for testing where fractionation of the expressed plant juice is being attempted. As mentioned above, it appears that the sheep is less susceptible than the cow to bloat on legumes.

"Feed lot" bloat In the United States, cattle are brought in from the range and "finished off" on a ration of hay and concentrates. After a period of weeks or months on this type of food a type of bloat known as "feed lot" bloat may develop. It was shown that this type of bloat could be induced on a diet devised by Smith et al. (1953). Jacobson & Lindahl (1955) have also produced the frothy "feed lot" bloat on a diet consisting of barley 61%, soyabean oil meal 16%, NaCl 1% and lucerne meal 22%. They found a definite increase in the incidence and severity of bloat over a period of eleven weeks on this ration. An advantage of this approach to the problem is that a diet of known and constant composition can be fed over a long period

whereas with pasture bloat the food composition is subject to constant change.

Special procedures have been used in elucidating particular points in ruminant physiology as applied to the bloat problem. Much research work has been carried out by artificially introducing large quantities of gas into the rumen (Cole et al., 1945; Clark, 1950; Weiss, 1953b; Nichols, 1954; Dougherty et al., 1955). Surgery and drugs (Weiss, 1953b) have also been used to study various aspects of the problem.

THE MECHANISM OF ERUCTATION IN RUMINANTS

Eructation in ruminants is of necessity a complex act on account of the anatomy of the organs concerned. The gas produced in the rumen and reticulum collects as a pocket in the dorsal rumen at a point rather higher than the cardia, the entrance to the oesophagus. The cardia is in fact normally below the level of ingesta. Eructation has therefore to be a coordinated process involving activity of the reticulum, rumen, and oesophagus. This results in the clearing of the cardia of ingesta, the bringing of the gas bubble forwards and downwards, and the opening of the cardia at the appropriate time to allow the passage of the gas into the oesophagus.

The stimulus initiating the eructation reflex is now generally believed to be stretching of the stomach wall (Weiss, 1953b; Iggo, 1955, 1956; Williams, 1955). From the work of Dougherty & Meridith (1955) in which eructation movements continued in a sheep whose rumen was completely filled with water under pressure, it appears that gas as such is not necessary. Stretch by any means will evidently suffice. If pressure is the only stimulus the receptors must be very sensitive for there is normally little change in the average pressure in the rumen between belches. Further, belching commonly occurs when the gas pocket is allowed access to the atmosphere through an open fistula, although Staflors (1926) believed that local pressure could still be a sufficient stimulus in these animals.

In the past it was thought that the belching reflex was similar to the regurgitation reflex and the stimulus was scratching of the stomach wall by roughage (Cole et al., 1942). However, it is clear from the work of Clark (1948), Weiss (1953) and Downie (1954) that two very different reflexes are involved. Weiss (1953b) found that during insufflation experiments under a given set of conditions eructation efficiency was inversely proportional to intra-ruminal pressure. He also kept an animal on succulent young lucerne tops for a month. In spite of the absence of roughage there was no adverse effect on eructation efficiency, ruminal motility or rumination. Johns (1954) found that with increasing pressure during the onset of bloat on clover, when according to the theory of Cole et al. (1942) there is insufficient roughage, frequency of eructation increased rather than decreased.

The location of the receptors has been given as around the cardia area (Williams, 1955, cows; Iggo, 1955, goats), and the posterior dorsal sac (Weiss, 1953b, sheep). Iggo (1955) has recorded nerve impulses from individual stretch receptors in the oesophageal groove region. He concluded that they were similar to those in the urinary bladder and were tension-recorders in series with muscle fibres. The pathway of the afferent fibres from these receptors was found to be the vagus. Nothing is known about

the centres involved in the reflex. There is ample evidence that the efferent pathway is also the vagus (Duncan, 1953; Iggo, 1956).

The motor events set in train by the nervous reflex have been investigated by a number of workers (Wester, 1935; Phillipson, 1939; Hoflund, 1940; Weiss, 1953; Dougherty & Meridith, 1955; Williams, 1955; Dougherty & Habel, 1955). The sequence of events was described by Dougherty and Habel who used cinefluorographical records of sheep insufflated with a mixture of carbon dioxide and methane. According to these workers, eructation is composed of the following motor events:-

(a) Two contractions of the reticulum,
(b) Contraction and raising of the rumino-reticular fold,
(c) General ruminal tonus or contraction forcing gas forward and downward into the relaxed and relatively empty reticulum,
(d) Contraction and dilation of the oesophagus,
(e) Relaxation of the two caudal oesophageal sphincters,
(f) Gaseous distension of the oesophagus,
(g) Continued closure of the cranial oesophageal sphincter retaining the gas in the oesophagus until the cardial and diaphragmatic sphincters close,
(h) Closing of the glottis causing a transient rise in intrapleural pressure which aids the oesophageal musculature in rapidly clearing the oesophagus of gas. This occurs through the relaxed cranial oesophageal sphincter.

Dougherty and his co-workers, who were the first to show the presence of the three oesophageal sphincters, (Dougherty & Meridith, 1955; Dougherty & Habel, 1955) consider that there are receptors involved in the reflex which can distinguish between gas pressure, which stimulates the cranial oesophageal sphincter to open, and fluid pressure which does not. This is important as it explains why foam is not belched out except in extremis. These workers also demonstrated that the mechanism is able to work despite overfilling of the rumen and reticulum with water. However, if the hind legs of the animal are raised so that the cardia is submerged to a greater depth, belching is impeded or prevented (Nichols, 1951; Weiss, 1953b). From the work of Cole et al. (1942), it appears that simple gas pressure will not open the cardia, but that belching will only occur as a consequence of the complete cycle of events. Nevertheless, from our own observations, it is clear that during insufflation gas may escape by eructation at the peak of normal backward rumen movement, immediately after the second reticular contraction. For this reason some caution must be used in accepting Dougherty and Habel's description of events which were observed during insufflation at a pressure of 30 to 40 mm. of mercury to stimulate the eructation reflex. Whether their observations on sheep can be applied in their entirety to cattle is uncertain until further observations have been made on this species.

Such a complex chain of events is clearly vulnerable at many points. It has been amply demonstrated that interference with the nervous arc, the efficiency of the rumino-reticular musculature or the patency of the oesophagus will impede or prevent belching. Thus a partial or complete section

or blocking of the vagi (Duncan, 1953; Weiss, 1953; Iggo, 1956) by which means conduction in both afferent and efferent fibres is prevented, will produce varying degrees of paralysis of the rumino-reticular musculature and can result in bloat (Weiss, 1953b). Chronic bloat can result from peritonitis involving the abdominal vagi. Depression of the brain centres by general anaesthesia produces a total paralysis of the stomach musculature and bloat may result if gas production is high enough. Interference with the efficiency of muscular activity by the use of drugs producing either hypomotility, e.g. cyanide (Quin & Van der Wath, 1938; Weiss, 1953b); atropine (Weiss, 1953b; Duncan, 1954); histamine (Dougherty, 1942; Clark, 1950; Weiss, 1953b; Dain et al., 1955); adrenalin (Dougherty, 1942; Weiss, 1953b; Duncan, 1954), or hypermotility, e.g. carbamyl choline (Weiss, 1953; Duncan, 1954); veratrine (Weiss, 1953b) will affect eructation and may result in bloat. Mechanical effects of overfilling and of raising the hindquarters have been mentioned. Obstruction of the oesophagus, e.g. by a piece of turnip, may result in acute bloat, while chronic bloat may result from partial obstruction, such as is caused by enlarged mediastinal glands following tubercular infection in cattle.

Demonstration that a particular stage in the eructation mechanism is vulnerable and that interference at that point will result in bloat does not prove that the point concerned is involved in the pathogenesis of clinical bloat. In the early stages of legume bloat it certainly does not seem that the eructation reflex is impaired for if anything the belching rate is increased (Johns, 1954; Hancock, 1954). In the most acute phase however, paralysis of the musculature due to pressure may supervene (Hancock, 1954; Williams, 1955).

THEORIES AS TO THE CAUSE OF BLOAT

Gas production

The most obvious conclusion that one comes to in observing a bloated animal is that the beast is producing more gas in its rumen than it can eliminate by belching. A theory of excessive gas formation was widely accepted until comparatively recently. However, the work of Cole et al. (1942, 1945) has made it clear that though there is a considerable difference in rate of gas formation from different feeds, it does not seem to be a factor in the aetiology of bloat. It is now generally agreed that when no other complicating factor is present the animal has no difficulty in getting rid of all the gas that could be formed (Cole et al., 1945; Quin, 1943). The amount of gas produced in the rumen on a particular food is roughly proportional to the amount of herbage ingested. It was found (Johns, 1954) that bloat occurred in animals with very different intakes and that dry matter intake of the severely bloated animals was on the average less than that of milder cases. In fact it was shown that the more severe the bloat the lower the intake which is a reflection of the fact that the onset of bloat caused the animal to stop eating.

It has also been assumed that it is the greedy eater that bloats (Cole et al., 1945) due to the rapid ingestion of too much succulent food. It was found that there was no relationship between rate of food intake and severity of bloat (Johns, 1954). Hancock (1954) in grazing trials with cows on

potentially dangerous grass found they they had relatively short grazing times with few periods of intense activity and showed a marked reluctance to graze.

Lindahl & Davis (1955) in "feed lot" bloat observed that the regular bloaters are prone to be slow and finicky eaters rather than greedy eaters. Many of the bloat symptoms develop before the animals finish eating and if marked enough the animals refuse to consume their full food rations. Therefore, over a period of time the total food consumption of the bloaters may be lower than for the non-bloaters.

Paralysis of the rumen musculature

A considerable amount of work has been carried out on the assumption that bloat is primarily due to an inhibition of the eructation reflex or to the paralysis or loss of tone of the rumen and/or reticulum. A large number of substances have been incriminated.

Cyanide Evans & Evans (1949) believed that cyanide was an agent in the production of legume bloat. Clark & Quin (1945) found no inhibition of eructation in their tests with cyanide while Weiss (1953b) found in sheep that small doses of potassium cyanide (150 mg.) inhibited contractions of the rumen. Introduced air could be eructated provided the rumen was not overfilled. Larger doses of cyanide caused complete inhibition of all mobility and eructation but at this stage the animals exhibited hyperpnoea and other symptoms of acute prussic acid poisoning. It appears evident that bloat incidence on clover bears little relationship to its cyanide content. Red clover used in the experiments of Newbold (1953) and Johns (1954) contained no cyanogenetic glucoside. Dougherty (1953) also pointed out that birdsfoot trefoil, which has a history of causing very little or no bloat, has 19 times as much cyanide as ladino clover, often the cause of the disorder. Ferguson & Terry (1955) failed to produce bloat in animals dosed with flavones with or without cyanide. It is possible that cyanide could add to the severity of bloat on white clover by causing some inhibition of reticular movements.

Flavones Ferguson (1948) at Jealott's Hill looked for the presence in bloat-provoking herbage of a substance which might interfere with the eructation mechanism or rumen muscular activity. He found an active principle in lucerne juice which inhibited the activity of isolated rabbit gut. The substance was identified as the flavone tricin (Ferguson et al., 1950). Ferguson & Terry (1955) carried out animal tests with quercetin, a flavone which has about the same muscle inhibiting power as tricin. Obtaining completely negative results, they concluded that it is unlikely that flavones play a part in the production of bloat.

Alkalinity and high ammonia levels in the rumen Clark & Lombard (1951) showed that the intravenous injection of alkali resulted in immediate ruminal paralysis. The dosing of alkali into the rumen produced a similar effect. It appeared that an alkalosis suppressed the ruminal centre. Weiss (1953) showed that the average eructation efficiency of six sheep had a remarkable correlation to the average CO_2 combining power of the blood and obtained complete rumen paralysis at pH 7·8. However, Ash (private communication) removed the rumen contents of sheep, titrated them with

sodium carbonate, returned them to the rumen but found no inhibition of ruminal contractions at a considerably more alkaline pH than 7·8.

When excess protein is present in the rumen together with a deficiency of sugars, the deamination process gives rise to ammonia. It has been assumed that a rise in ruminal pH results. However, Johns (1955) has shown that, on pasture with protein levels of 35% of the dry weight, ammonia levels of over 100 mg. NH_3N/100 ml. occurred in the rumen of sheep without the pH rising above 6·5. In measurements of the pH of rumen contents of cows on bloat-producing red clover the pH was always below this when bloat occurred. Hale & King (1955a) in a study of urea toxicity in ruminants, found that bloat occurred when urea was administered orally in doses that were fatal or near fatal. They believed that the toxicity was due to the formation of ammonium carbamate. Hale and King consider that since immature legumes are high in non-protein nitrogen, fermentation would favour the production of ammonia and carbon dioxide. They consider that death during acute bloat in cattle may be actually due to the absorption of ammonia carbamate or some related nitrogen compound.

However, the author, on feeding winter grass, found that high ammonia levels can be maintained in the rumen of sheep without any signs of bloat or toxicity (Johns, 1955).

Histamine A number of workers have postulated that the central feature of bloat in ruminants is a paralysis of the rumen musculature by histamine or a histamine-like substance. The agent is considered to be present in the rumen ingesta or to be released systemically as a result of an allergic reaction.

Dougherty (1942) showed that the intravenous injection of histamine caused ruminal paralysis and found large amounts of histamine in the ruminal ingesta of cows that had died of bloat. The histamine content of the ruminal ingesta was increased on a high protein diet. Clark (1950a) also showed that histamine given intravenously caused rumen paralysis while Petersen (quoted by Cole *et al.*, 1945) claimed to have produced marked bloat by this procedure.

Kerr & Lamont (1946) and Lamont (1946) noted a high incidence of bloat following the second dose of a trichomonal antigen. They postulate an allergic reaction in which histamine release is involved and claim success in relieving the bloat symptoms with 3–5 ml. dose of adrenalin. This medication was also successfully used in the treatment of field bloat on clover, green corn, and young green grass.

Treatment of bloat on the basis that it is mediated by histamine has apparently been successful in the hands of some workers. Carmichael (1951) states that: "Certain cases of bloat ... on clover have responded well to anthisan [mepyramine maleate], especially those associated with a sudden change to a high protein diet such as clover." Sweet *et al.* (1949) used another antihistaminic, pyribenzamine, in a case of anaphylactic shock in which bloat was present.

In the treatment of legume bloat Johns & Reid (1954) and Johns (1954) found that the use of adrenalin or an antihistaminic was dangerous and increased its severity. This is in line with the findings of Dougherty (1942) that adrenalin at the level recommended causes marked depression in both strength and frequency of ruminal contractions in cattle. While these

treatments may be of use in other types of bloat, they appear to be of little use in the disorder caused by clover ingestion.

Toxic factors in rumen ingesta It has been claimed that the digestion of legumes in the rumen causes the excessive production of toxic gases such as carbon monoxide and hydrogen sulphide which could play a part in the pathogenesis of bloat. However, there is no evidence that these substances are in any higher concentration in bloated than in normal animals (Cole *et al.*, 1945). It has been stated that the hydrogen sulphide content is higher in the rumen of animals feeding on legumes due to their protein content. The work of Lewis (1954) and Anderson (1956) has emphasized the importance of sulphate as a source of rumen hydrogen sulphide. The indications were that the ruminant can deal with relatively large amounts of H_2S without showing any toxic effects.

Dougherty & Cello (1949) in studying acute indigestion in sheep found a toxic substance in rumen ingesta which inhibited rumen mobility and depressed the blood pressure of cows and goats. The factor was also present in the ingesta of two cows that died of bloat.

From the feeding experiments of the author (Johns, 1954) in the production of bloat it did not appear that any marked toxicity was present. Bloating twice a day for many days did not reduce appetite nor affect the appearance or vigour of the animals. Their appearance and behaviour was normal a short time after recovery from severe bloat. Moreover, when the same food produced bloat in some animals and not in others the non-bloaters consumed more of it without any signs of toxic effects.

Anti-choline esterase Interest in choline esterase arises from the fact that it is responsible for the hydrolysis of acetylcholine which is the chemical mediator of the parasympathetic nervous system. The enzymatic hydrolysis of acetylcholine is strongly inhibited by the alkaloids eserine, prostigmine and atropine. The esterases are irreversibly inhibited by such agents as disopropylfluorophosphate which can produce bloat in sheep and goats. When choline esterase is inhibited, acetylcholine synthesis continues and the concentration rises. An excess of acetylcholine may cause a muscular spasm.

Heath & Parke (1953) found in white clover an irreversible choline esterase inhibitor. This was not always present and the authors consider that the clover may in some cases produce signs of anti-choline esterase poisoning as well as cyanide poisoning. Alder (personal communication) of the Grassland Research Station, Hurley, in conjunction with the Veterinary Laboratory, Weybridge, compared levels of choline esterase in blood of cattle on winter feeding in yards with animals grazing on bloat-producing pastures. Following grazing there were slight depressions of choline esterase activity in 7 out of 10 cattle, but these could not be correlated with bloat.

The physical nature of the food

The lack of roughage or scabrous deficiency theory The most potent bloat-producing pastures are relatively succulent and low in roughage, and it has been suggested that lack of roughage is an important factor in the pathogenesis of the condition. Two distinct theories have been put forward. According to Cole and his associates (1942) roughage is necessary to stimu-

late belching, and deficiency of roughage results in decreased belching efficiency. This theory has been widely held in the U. S. A. More recently, Clark & Weiss (1952a) in South Africa have suggested that the role of roughage is to stimulate salivary flow, ensuring a watery non-viscous rumen ingesta. They consider that bloating is due to foam formation in a viscid medium insufficiently diluted with saliva.

The main evidence for these theories is the prevention of bloat by feeding of roughage before grazing on potent pasture. In the American work, it was found that bloat could be prevented by feeding 10 or 15 lb. of Sudan grass hay to lactating dairy cows pastured on legume pasture during the day. Cole et al. (1945) emphasized that Sudan grass hay was the only roughage studied which was completely effective and it had to be supplied overnight if the cows were to eat enough to be protected.

Although a considerable amount of Sudan grass hay does give protection there is little evidence of a correlation between bloating potential and the degree of scabrousness of the food. In feeding experiments with red clover (Newbold, 1953; Johns, 1954) bloat was experienced at all stages of growth, even when seed had set. When no bloat was being experienced in the area irrigation of young red clover failed to make it toxic although the same food produced bloat subsequently when it was more mature. Further, bloat was produced on red clover hay of 88% D. M. (Johns, 1954), and it is not uncommon for bloat to occur on lucerne hay in the U. S. A. (Marston, 1953). In observations on 1575 cases of bloat, Barrentine et al. (1954) state: "The moisture content or lushness of growth of the clover did not appear to be a factor in the incidence ... of bloat." It is general farm experience that though the most severe bloat generally occurs on the more lush food such food does not necessarily produce bloat.

Clark & Weiss (1952a) demonstrated the existence of a salivary reflex initiated by stimulation of the mucous membrane of the forestomachs. Weiss (1953a) found that bloat in sheep occurred immediately on feeding succulent, leafy lucerne in the preflowering stage of growth, and was caused by frothing of the thick, viscid rumen ingesta. When mature stalky lucerne was fed rumen ingesta immediately reverted to a watery consistency even in the absence of drinking water and bloat ceased. This rapid reduction in viscosity was attributed to reflex stimulation of the salivary flow by the stalky food.

There is no doubt that the amount of saliva secreted is directly related to the condition of the food. Balch (1954) has shown that per 10 lb. of food a cow secretes when fed with hay 45–55 lb. of saliva, 12–15 lb. with ground concentrates and 7.5–10 lb. with grass. It is therefore difficult to reconcile the lack of saliva theory with the production of bloat on hay. Though Weiss's (1953a) correlation of ingesta consistency index with bloat on lucerne is of considerable interest, Jacobson & Lindahl (1955) found no such correlation using a similar technique in a study of "feed lot" bloat.

Ferguson & Terry (1955) tested the effect of synthetic and natural saliva on the production of bloat in sheep and found no reduction in bloat on their addition. One sheep received 2.5 litres of lucerne juice and 750 ml. of cow's saliva while two control animals were given 2.5 litres of juice alone. All three sheep became severely bloated. The fact that a number of workers have produced bloat on legume juice would indicate that a lack

of liquid is not necessary for the production of the condition. Further, the role of saliva itself needs clarification since bovine saliva contains a very powerful surface active agent and is capable of forming strong foams (see below).

From the strength of the contrary evidence, it appears fairly clear that factors other than fibrousness or maturity are involved in pasture bloat.

Density of the food The theory that a mechanical blocking of the cardiac orifice with rapidly ingested food has been put forward with various modifications by a number of investigators in the past (Cole et al., 1945). Nichols (1954a) has a new approach to the part played by the density of the food. He considers that, because of their high density, legumes entering the rumen sink to lower levels than do less dense foods. The subsequent decrease in density of the fluid itself due to gas bubble formation causes the food to descend still further, so that the main fermentation tends to take place in the ventral portion of the rumen. If conditions are right, foam will then form throughout the rumen. The sinking of the food causes the liquid level in the rumen to rise, and it is this, together with the foaming, that interferes with eructation during bloat.

Observations at this laboratory do not confirm Nichols' views. In animals bloating on both red and white clover the ventral portion of the rumen was found to be almost completely liquid with little solid material. This liquid showed little foaming capacity. The main collection of small bubbles was in the dorsal and middle regions and in the posterior ventral sac, which were the regions where active fermentation was proceeding as judged by pH, volatile acid and ammonia concentrations. The foaming material at the top of the rumen was as much as .5 of a pH unit lower than the ventral portion and had on occasions almost double the concentration of volatile fatty acids.

As with the roughage theories, it is hard to reconcile Nichols' theory with the production of bloat on hay. Further, it seems unlikely from the work of Dougherty (1953) and Weiss (1953) that a blocking of the cardia by a raised liquid level is the primary cause of bloat. Owing to the efficient clearing of the cardia by reticular contractions, considerable difficulty has been experienced in preventing eructation by raising the liquid level in the rumen and reticulum.

Foaming or surface tension theory

General Dougherty (1953) has stated: "This theory embraces any material which will change surface tension so that the gases of fermentation will tend to accumulate in countless bubbles throughout the ingesta instead of rising to the top and collecting in a gas pocket above the ingesta."

That frothy bloat does occur is widely accepted but how important it is in relation to the free gas type is an open question. The tendency has been to differentiate the two types sharply. It may well be that the apparently different types of bloat differ only in the quantity of free gas in the rumen. It seems most likely that where gas is being retained in a foam, the stability of the foam and rate of gas production will determine how much is retained within the liquid phase and how much escapes to form a free gas pocket (Weiss, 1953b; Johns, 1954).

Lindahl & Davis (1955) in "feed lot" bloat found that although the cause of bloat appears to be the formation of very stable frothy ruminal contents, they always found a pocket of gas and at times a considerable amount of free gas in the rumen. They were able to release enough free gas with a stomach tube to produce a marked reduction in ruminal distension. However, if the fistula cannula was opened rapidly, frothy ruminal contents would pour out.

The presence of a free gas pocket in bloat on red clover is demonstrated by the slow leak of gas around the fistula in fistulated cows. In the author's experience this occurs even when considerable distension of the rumen is present. The increase of the amount of free gas is obvious following drenching with oil, by the increase in the amount of gas escaping via the fistula leak.

It has been the experience of Lindahl & Davis (1955), and of the author that a marked difference exists in the physical state of the rumen contents between bloating and non-bloating animals. In a non-bloating cow the rumen contents on removal from the rumen will separate into two distinct phases, or if squeezed through muslin, will give a liquid layer with a small amount of foam on the surface. On the other hand the rumen contents from the upper portion of the rumen of the bloating animal appear as a homogeneous frothy mass. If this is squeezed through muslin a column of foam is produced with no liquid layer. This foam may remain for many hours with a liquid layer only slowly draining from it.

The degree of bloat appears to be associated with the stability of the foam.

Foaming

Theory There are two broad groups of foaming materials:-

(A) Solutions of soaps and detergents in which the surface tension is considerably lower than that of water and in which surface tension is related to the stability of the foam. In general the foams formed are not particularly stable and are non-viscous.

(B) Those exhibiting a strong superficial viscosity which includes saponin solutions and proteins in the presence of suitable metal salts. These yield foams of great stability and at the same time exhibit fairly high surface tension values which show little correlation with foam stability.

The surface viscous and the non-viscous type are mutually incompatible. If a soap and saponin solution are mixed, the soap having the lower surface tension causes displacement of the saponin from the surface layer. This reduces the viscosity of the system and gives a more unstable foam. For this reason detergents have been found to give relief in bloated animals (Nichols, 1954a). In general antifoaming agents act by displacing the foaming substances from the surface of the foaming liquid and by providing a non-foaming surface layer.

Technical methods The difficulty in studying foams is that research has not been able to produce an absolute value independent of apparatus and method used, that can be a measure of foaminess of a liquid. It has been suspected for a long time that the stability of a foam is connected in some way with surface tension, and though this is now known generally to be true there are a number of other factors involved.

There are various methods of studying foams, the most usual being

to measure the height of a column of foam produced in a cylindrical vessel by passing a stream of gas in the form of fine bubbles through the liquid. This, however, gives very little information as to the stability or strength of the foam, which bears an inverse relationship to the size of the bubbles.

Hungate et al. (1955) found some correlation between the degree of bloat and foam production by ingesta incubated in large Warburg vessels. Jacobson & Lindahl (1955) observed that the rumen contents of cattle on a bloat-producing diet after removal from the rumen increased in volume with time, due to the entrapment of gas in small bubbles throughout the rumen contents. They measured the "ingesta volume increase" by placing an aliquot 200 ml. of freshly drawn rumen contents in a 500 ml. cylinder and incubated at 39° C. After an hour the increase in volume was recorded. The stability of the froth produced was measured by vigorous stirring of the contents of the cylinder 72 times and a second reading was recorded as the stable "ingesta volume increase". Lindahl & Davis (1955) modified this to incubate 200 ml. of rumen contents sampled before feeding with 3 g. of glucose. There appeared to be some correlation with the resulting "ingesta volume increase" after an hour's incubation, and the degree of bloat when the animals were subsequently fed.

Mangan (unpublished) has used a method of bubbling carbon dioxide through a sintered glass sparger and producing a column of foam at 37° C. After the foam has reached a definite height in the column, its strength is determined by the rate of fall through it of a perforated disc of some 120 g. in weight. This method has yielded useful information on the foaming properties of various plant constituents.

Foaming system in the rumen The foaming substances present in the rumen appear to be of the viscous type of which there are several possibilities.

Saponins — A number of people have postulated that saponins are involved in the aetiology of bloat. Lindahl et al. (1954) appear to be the first to have produced bloat on a saponin isolated from a pasture legume. A considerable amount of work has been carried out by the Western Regional Res. Lab., U. S. Dept. Agric., Albany, Calif., on lucerne saponins and a number have been found to be present in the plant. Colvin et al. (1955) found that the dosing of 100 g. of lucerne saponin dissolved in a litre of 5% glucose caused death in one and a half to two and a half hours. Post mortem examination indicated that death was associated with an intense submucosal and subserosal haemorrhagic condition of the gastro-intestinal tract. Foaming bloat was also observed. It was observed that rumen motility was markedly depressed by either 25 g. of lucerne tops or 12.5 g. of lucerne saponin.

It appears doubtful whether the condition resulting from the dosing of saponins is similar to that obtaining during their slow release from the plant material by microbial digestion. Confirmation of the depressant effect of lucerne tops on rumen motility is also desirable. No such effect has been observed in bloat on red clover in a large number of direct and indirect recordings (Reid, unpublished).

Ferguson & Terry (1955) prepared saponins from lucerne juice that was markedly bloat provoking. However, 22 g. in aqueous solution given to a sheep did not produce bloat while the same amount in lucerne juice

produced only mild bloat. These workers in fractionating lucerne juice found that in the juice freed from chloroplastic material the bloat promoting factor was not precipitated by 75% alcohol, was non-ionic and was not absorbed on resins or charcoal.

One of the present limitations in testing the role of saponins is that there are no simple routine tests available to determine whether there is a correlation between saponin level in the herbage and its bloating potential. Ferguson & Terry (1955), however, found that the frothing capacity of juice from fresh lucerne expressed from a triple roller mill gave a fair indication of its bloating potential. They also found a loss of foaming capacity on drying the herbage, which suggested either a breakdown of a frothing constituent or a change in the physical state of a constituent. This may be of considerable importance as the work of Dr. C. R. Thompson and coworkers at the Western Regional Research Lab., U. S. D. A. on the isolation of saponins from lucerne has been carried out on dried material. They found in preliminary analyses that the saponin content of lucerne vaied between 0.2 and 1.8% on a dry matter basis, with considerably more saponin in the leaves than in the stalks (Thompson, personal communication).

Protein — It was postulated by Johns (1954) that protein or its degradation products may play some part in bloat. It would not be expected that protein would be released rapidly enough from ingested material for it to play the major role in the very rapid onset of bloat as is sometimes experienced. However, it was found (Mangan, unpublished) in an animal without food for 24 hr. that the soluble protein nitrogen in the rumen liquor increased from 3.9 mg. N before feeding to 19.3 mg. per 100 ml. in 1 hr. from the commencement of eating red clover. What proportion of this protein nitrogen was contributed by the saliva was not determined. Mangan found that in the presence of salts the red clover cytoplasmic proteins at 20 mg. protein N/100 ml. formed a very stable foam with a maximum stability at pH 6·0. In studies of the conditions in the rumen at the onset of bloat it was found (Reid, unpublished) that the pH was in the region of 6·3. As the fermentation proceeds it dropped to below pH 6, and hence remained in the region where the protein foam is stable. It appears that the conditions in the rumen are suitable for protein to play at least a minor role in the production of a stable foam.

Saliva — A medium by which the animal could contribute to the foaming properties of the rumen ingesta is through its saliva.

Reid & Huffman (1949) found that the surface tension of bovine saliva was low compared with water and concluded that it must contain a potent surface active agent. Mangan (unpublished results) found that mixed bovine saliva formed a stable foam even when diluted by a factor of ten so that it could make a substantial contribution to the foaming properties of the rumen ingesta. It may be that there is some difference in chemical composition of saliva from bloating and non-bloating animals as well as difference in quantity. Phillipson (private communication) found considerable animal differences in saliva flow response to rumen stimulation. It appears to the author that it is important to work with the mixed saliva in tests of this hypothesis rather than parotid saliva as has been done by a number of workers.

Bacterial polysaccharides — An hypothesis involving a change in the nature of the ingesta to explain at least in part the cause of frothy bloat has been advanced by Hungate et al. (1955) for legume bloat, and by Jacobson & Lindahl (1955) for "feed lot" bloat. Hungate et al. (1955), in considering the differences in the behaviour of the rumen ingesta from "bloaters" and "non-bloaters" on the same food, thought it questionable whether these could be explained by differences in rate of fermentation of plant material or of released saponins. They put forward the idea that the ingesta of regular "bloaters" contained a greater proportion of slime-producing micro-organisms. This microbial slime could then account for the formation of a stable foam.

A similar hypothesis was put forward by Jacobson & Lindahl (1955) for "feed lot" bloat, where the adaptation to the bloating condition may take many weeks on the same food. This adaptation was shown by Jacobson and Lindahl to be accompanied by a gradual increase in the number of slime-producing organisms present in the rumen.

The sudden onset of pasture bloat would appear to rule out any slow adaptation of the rumen flora, but the fact remains that there is a definite difference in the physical properties of the ingesta of bloating and non-bloating animals, or between the ingesta of the same animal in the two conditions. It has recently become clear that extracellular bacterial polysaccharide is abundant in the rumen (Hobson & McPherson, 1953). It has been found (Oxford, 1955) that certain bacteria from the rumen only form extracellular polysaccharides when a disaccharide or higher polymer is fermented, while dissimilation of the simple sugars gives no increase in extracellular polysaccharide. It appears that differences between the sugar fractions of bloat potent and non-potent clover should be explored.

Antifoaming agents — There is the possibility that instead of an increase in foaming agents in the plant, a decrease of natural antifoaming agents occurs. This is at present being investigated (Mangan & Fraser, unpublished). It is of interest that in the experiments of Reid (1956b) in the control of legume bloat by oil spraying, control is achieved with 2–3 oz. of oil per day per cow. This represents only about one-quarter of the daily intake of fat of the animal. Evidently microbial digestion releases this fat more slowly than it releases the foaming agents.

Summary of the foaming theory

It seems definite that the foaming in the rumen is due to agents of the saponin and protein type but the system appears to be a complex one with perhaps polysaccharides and mucoproteins playing a part, while the balance of foaming and antifoaming agents may be an important factor.

The determination of the substances involved in the production of frothy bloat is not simple since besides a plant factor the rumen must be in the requisite condition for the trouble to manifest itself. Lindahl et al. (1954) and Ferguson & Terry (1955) found that the animal must be on a legume diet for positive results in testing out saponin or plant fractions for their bloat producing qualities. Sheep on grass did not respond. It appears therefore that the condition of the animal at the time of feeding bloat provoking herbage is as important as the nature of the herbage itself if bloat

is to result.

THE CAUSE OF DEATH AND PRESSURE IN THE RUMEN

The cause of death in bloat is not known. There are two main theories, both of which have been inadequately investigated:

(1) mechanical interference with respiration and blood flow,

(2) toxic factors diffusing from the rumen into the blood.

These have been discussed by Cole et al. (1945) and Dougherty (1953). It may well be that both play some part but this aspect of the problem is probably the one about which we have the least concrete information. It is probable that information obtained using sheep will have to be applied with considerable reservation to cattle.

The relationship between gas pressure in the rumen, its effect on the animal, and the severity of bloat have also been inadequately investigated due mainly to the experimental difficulty of examining field bloat. Kleiber (1945) developed a tympanometer for measuring the pressure in the rumen by placing the instrument against the bulging flank of the animal. Kleiber found that a cow is markedly bloated at 30 mm. of mercury and recorded a pressure of 60 mm. in severely bloated animals which would probably die if not promptly relieved. However, in the hands of other operators the tympanometer has not been found to give as reproducible results as obtained by Kleiber and his students.

Dougherty (1953) quotes figures of pressures of 72 to 75 mm. of mercury recorded in 3 bloated cows that had recently died. Nichols has insufflated sheep to between 262 and 524 mm. of mercury which appear extraordinarily high in comparison to the bloat figures from cattle. Dougherty et al. (1955) in a study of the physiological effects of insufflation of the stomach of sheep, found a marked difference between individual animals in their tolerance to the same gas insufflated at the same pressure. In an experiment using a 40% methane, 60% carbon dioxide mixture, sheep A collapsed within 3 min. with a pressure of 40 mm. whereas sheep B resisted a pressure of 80 mm. for 5 min. before collapse, in both cases recovery was obtained by release of intraruminal pressure. Increased intraruminal pressure caused sharp rises in arterial and venous blood pressures and cerebrospinal fluid pressure. Dougherty also found that the composition of the blood gases was rapidly and markedly affected by the intraruminal pressure. Oxygen utilization was decreased, as was the pulmonary circulation time. It is to be hoped that work along these lines on bloated animals should give a lead to the cause of death.

THE PREVENTION AND TREATMENT OF BLOAT

Pasture management

The grassland economy of New Zealand depends primarily on clovers as virtually the sole source of nitrogen in high production pastures and they are therefore an essential element in the association. Hence any tendency on the part of the farmer to refrain from the use of clovers on account of their bloat-producing qualities will eventually lead to a lower grassland production.

As we cannot afford to dispense with clovers in dairy pastures, the more feasible solution lies in the use of grass dominant pastures. In this, the aim is to reduce the intake of potent clover to safe levels by diluting it with harmless food. The use of mature, grass dominant pasture is probably the best single measure of protection we know of at the present time. One difficulty has been that increasing maturity of the grass component of the sward is associated with decreasing palatability. In New Zealand, the use of short-rotation ryegrass with its vigorous spring growth and its palatability when mature has been advocated. However, the establishment and maintenance of such a grass dominant pasture requires a high level of fertility which can only be built up over a period of years. It seems to be widely agreed (Dougherty, 1953) that pastures containing less than 50% clover may be considered safe. The safe proportion however will always be difficult to define as it will depend on the potency of the clover, the palatability of associated grasses and the selective grazing habits of the animals. Troughton (1955) produced bloat in his grazing trials with clover levels as low as 33% of the sward and this also occurs under New Zealand conditions.

A common farm practice based on the dilution method is to graze the cows for a short period on a break of dangerous pasture and then remove them to safe food such as rough pasture, autumn saved pasture, crops or silage. This system entails considerable risk particularly if the animals are fed the dangerous pasture when hungry.

Grazing control

There are two main objectives in measures involving grazing control: firstly to restrict selection of clover, and particularly the succulent clover tops, which are believed to be more potent than the remainder of the plant; and secondly, to alter the grazing pattern and reduce the rate of intake to such a level that the animal is able to cope with potent material.

Control of selection has been achieved by cutting the herbage and stall feeding the animals or allowing them to pick it up themselves. This procedure may reduce moderate bloat, but more than 2,000 cases of bloat have been produced at the Plant Chemistry Laboratory, New Zealand, over the last few years with cut red clover, carted and fed to animals. Moreover Newbold (1953) found that when stalks and leaves of red clover were fed separately bloat occurred on both.

A second method based on the concept that control of bloat could be achieved by eliminating selective grazing and by making the animal eat the whole plant is the use of the electric fence (Sears, 1953). Under this system the herd of cows is intensively grazed on a number of strips or "breaks" of pasture during the day. Though this may reduce the number of cases of moderate bloat, it cannot give full control of severe bloat without a reduction of food intake, with consequent loss of milk production (Reid, 1955). This system has a high labour cost and may lead to overgrazing of pastures. However, the electric fence is of considerable aid in the utilization of pasture which has been allowed to mature as it permits the efficient feeding off of tall pasture which would otherwise be partly wasted by trampling underfoot.

Hancock (1954) attempted to change the grazing habits of the cows. Arguing that bloat may be due to the period of intense grazing after milking, it seemed that a measure of prevention could possibly be achieved by offering the cows one half of their daily allocation of grass before the morning milking and the other before the afternoon milking. However, as found also by Johns (1954), the oft-stated opinion "bloat is caused by greedy eating of dangerous pasture" was not substantiated and the method showed little promise. Hancock also tried a system of "on" and "off" grazing to break up the grazing pattern. This showed some promise but was time-consuming and had other drawbacks. In general it was found that irrespective of the number of "breaks", break grazing with or without restriction gave no effective control of bloat.

Feeding of roughage

The control of bloat by prefeeding with hay has been mentioned earlier. The fact that Cole et al. (1945) have stated that it must be fed overnight to be effective, may have significance in that the condition of the rumen contents could be altered during this period. Feeding of hay just before turning stock out to pasture does not appear to be a reliable method for control on dangerous pasture.

It has been found by Cole (see Marston, 1953) that the rate of gas production on a diet of silage alone is very low as one would expect since silage has already undergone fermentation in the stack or pit. Whether or not prefeeding of silage will decrease the total amount of gas production sufficiently to prevent bloat when pasture is subsequently fed, will obviously depend on the amount of silage fed. There appear to be no controlled experiments in the literature on the use of silage as a prefeed in bloat control.

Antifoaming agents

The use of antifoaming agents appears to be a logical method of preventing frothy bloat. It was found in stall fed animals (Johns, 1954; Reid & Johns, 1956) that frothy bloat could be prevented by administration of antifoaming agents (approx. 2 oz.) before feeding on clover. These agents were administered as drenches or mixed with small amounts of dry food and gave complete protection from bloat for the succeeding 2 hours. It had been hoped that such a measure would protect the animals from morning till evening milking but even increasing the dose did not give protection consistently for this period. Hungate et al. (1955) had a similar experience. They dosed ten steers with 20 g. of methyl silicone just before grazing. Seven animals did not bloat in the grazing period following administration of the silicone. All steers bloated at the next grazing period.

It appears that the antifoaming agents used are lost rapidly from the rumen and hence the only reliable method would be to have a continuous intake with the food unless a more insoluble agent can be found. Clark & Weiss (1952b) in South Africa suggested spraying pasture with a weak solution of a proprietary antifoaming agent. Reid (1955) in New Zealand has successfully developed a method of pasture spraying for bloat control using emulsified vegetable oil sprays, particularly peanut oil.

It has been found (Johns, 1954; Reid & Johns, 1956) that the vegetable

oils, whale oil, emulsified tallow, medicinal paraffin, vegetable turpentine and "Avlinox" (a ricinoleic acid derivative) proved effective in the prevention of bloat of stall fed animals. The silicones were rather erratic in their behaviour compared with the oils. As the spraying of pasture may have to continue for some weeks it was important that the agent chosen should show no immediate or cumulative toxicity to the animal or affect the quality or quantity of milk and butter produced. Moreover the cost of the material employed should be reasonable.

The bland vegetable oils appear to have negligible toxicity and have the added advantage of being digested. There are however reports that the addition of corn oil and soya bean oil depress milk fat production (Allen & Fitch, 1941; Hale & King, 1955b). Also the vegetable fats, because of their absorption in the blood stream affect the properties of body fat and butterfat. McDowall (1955) gave results of a trial of various oils in which 300 ml. per cow were given night and morning. Linseed oil, soya bean oil and whale oil all had undesirable effects on the quality or flavour of the milk and butter. Peanut oil and a tallow emulsion had little effect on the flavour and characteristics of the butterfat, while none of the drenches caused a significant change in the yield, or in the fat and solids-not-fat content of the milk.

"Avlinox" and the silicones have low toxicity, but both are too expensive for the spraying technique, while the latter appear to be somewhat variable in their effectiveness.

Liquid paraffin and other mineral oils pass through the alimentary tract undigested and have a laxative effect. Isolated use is not harmful but continued use is known to interfere materially with the absorption of carotene, vitamins D, K, and calcium and phosphorus (Sollmann, 1948). Reid (unpublished) found in dosing cows with 75 ml. paraffin twice each day for four weeks that there was a marked fall in blood carotene and butter tocopherol and carotene levels.

Turpentine causes marked taint which may appear in the milk within a few minutes. Further, it is toxic and causes marked irritation in various organs and depresses the appetite of the grazing animal for a day or two.

The most suitable substances of those considered here for bloat control appear to be emulsified tallow and peanut oil. Tallow has the disadvantage that it is rather difficult to emulsify though Reid (1955) and Geddes (personal communication) have both used it successfully in bloat control.

Reid (1955) has completely controlled bloat on clover grass pastures by spraying with emulsified peanut oil at the rate of 2 oz. per cow per day. Cream was also found to be effective. The area required for the day's food was confined by an electric fence and sprayed. In a trial (Reid, unpublished) on red clover dominant pasture dodecyl benzene sulphonate was included for comparison and the results are shown in Table 1.

The one case of bloat on the oil-sprayed area was only mild in character. It will be noted that even with large doses of detergent (25 g.) bloat still occurred though the severity is reduced. All cows requiring to be drenched received oil and as all responded to the treatment the bloat was evidently all of the frothy type.

Spraying by using a boom spray mounted on a tractor has been used quite widely in New Zealand to control bloat during the 1955–56 dairy season,

TABLE 1

Group	Treatment	Total No. of cows	Cases of bloat	Removed for safety	Requiring to be drenched
Control	Nil	21	14	12	8
Oil	Emulsified peanut oil sprayed at 1½ gal. oil/acre. Intake per cow 2 to 3 oz. oil	20	1	-	-
Detergent dodecyl benzene sulphonate	Area sprayed at 1 lb./acre. Intake of detergent per cow ⅕ oz. A. No treatment before entering sprayed area	10	7	5	4
	B. Drenched 25 g. of detergent before entering sprayed area	11	5	-	-

and farmers in general are well satisfied that the cost of the method has been covered by the increase in milk yield achieved when compared with bloat conditions when no such control measures have been applied.

Geddes (personal communication) has used the technique on irrigated pastures in Australia. He states: "After grazing out herds on stands of almost pure clover for three years with impunity we struck trouble with bloat. Feeding hay had no effect nor did mowing or wilting for up to 24 hr. All the latter did was to reduce milk yields. Spraying with peanut emulsion gave us complete control. Then after 10 days of immunity from trouble we discontinued spraying to check whether the clover was still toxic. We had 26 cases out of 59 cows within an hour. That afternoon we resumed spraying and again were able to graze this sward without trouble!" Geddes also used tallow emulsion at the rate of 2 oz. of fat per cow per day and obtained equally good control.

One interesting effect which Geddes as well as New Zealand workers noticed is that when feeding bloaty pasture the animals become most restless and difficult to handle in the milking bail even though not actually bloating. When the pasture has been sprayed they quickly quieten down and milk yield rises.

Another method of bloat control has been to use oils on water troughs and this has met with moderate success in the field.

In attempts to prevent "feed lot" bloat Lindahl & Davis (1955) found up to 150 ml. of cottonseed or corn oil, given before feeding, ineffective. Liquid methyl silicone preparations given at twice the recommended therapeutic dose did not prevent an animal from bloating. It appears that the foam formed in the rumen during "feed lot" bloat is considerably more

stable than in pasture bloat. However, it may well happen that such severe bloat may be encountered on occasions on pasture that antifoaming agents at present used will not give complete control.

Ferguson & Terry (1955) state: "Many methods of reducing the risk of bloat in grazing animals have been advocated — hay feeding, reduction of drinking, controlled grazing, wilting of herbage, etc. — but none are certain preventatives of bloat in susceptible animals grazing leguminous herbage which is at a particular bloat-provoking stage of growth." While agreeing with this, the author considers that we have in oil spraying a method which though of little use in isolated cases of bloat can be successfully used to control the ailment economically where particularly potent clover has to be fed to a dairy herd. It has the advantage of allowing cows to stay on potent pasture and so permit other potent herbage on the farm to mature.

TREATMENT

The value of antifoaming agents in the treatment of bloat depends on the proportion of cases that are of the frothy type. Clark (1948) believes that most cases in South Africa are of this type or partially so. Our experience in New Zealand on the use of pasture spraying to control bloat is that the large majority of cases respond to this treatment. The agents used in the previous section for the prevention of the ailment have also been successfully used in relieving bloated animals by drenching. Such commonly used substances as turpentine and certain phenolic compounds have also been shown by Clark (1948, 1950b) to owe their efficacy to their antifoaming properties. Quin et al. (1949) were successful in the treatment of bloat with a methyl silicone preparation, though the use of collateral treatment makes it difficult to judge the efficacy of the method. Reid & Johns (1956) have found that the silicones at present in use are not as reliable as the vegetable oils.

With a number of remedies such as "Avlinox" and the silicones it has been recommended that they can be given by mouth or by injection through the flank directly into the rumen. There appears to be no evidence as to the relative effectiveness of the two routes.

Nichols (1954a) states that the administration of some of the common household detergents and surface active agents cleared the rumen of froth. Our experience has been that they are neither as reliable nor as effective as the vegetable oils.

Antifoaming agents are useful in cases where the animals are not in too distressed a condition. However, as was stated by Barrentine et al. (1954): "A trocar or knife appears to be the only treatment of value in an acute case." If only a small gas pocket is present a considerable incision may have to be made to remove solid material which blocks up a small hole. The usefulness of an antifoaming agent through the trocar is problematical if the animal is in such a condition as to warrant the employment of this instrument, at which stage speed is the essential requirement if the animal is to be saved.

The use of antihistaminics such as suggested by Kerr & Lamont (1946) was found by Johns & Reid (1954) to be definitely dangerous with frothy bloat, but antihistaminics have a place where an allergic reaction is involved.

In cases where the bloat is chronic, the use of such treatments as antibiotic therapy (Chivers, 1954) or calcium therapy (Rydell, 1955) may be indicated. There appears at present to be no logical basis for their employment in acute cases.

The use of such methods as standing animals uphill, a gag in the mouth or driving animals around the field has a number of adherents but is not reliable.

We can complement our findings with a quotation from a paper by Beddows (1952) on writings on hoven in cattle, 1716–1827:

"Richard Peters writing in the Agricultural Magazine for October 1807 (Vol. I) on hoven cattle, pleads that farmers should not be prejudiced against clover, "this great and extensive improvement. ..... any succulent or juicy food, if moist with rain or dew, has a capacity to generate air which by its expansion in the animal produces hoving. Lucerne, pea-vines, green Indian corn plants, and buck-wheat have under my own observation occasioned this destructive calamity.'

"..... he ..... gives four remedies:-

(1) 'Immediately stab the animal on the left side, behind the hind rib and the hip bone, not too near the latter, with a pen (or other small) knife.

(2) 'A beast has been relieved by violent eructations, on the tongue's being suddenly and forcibly drawn out.

(3) 'Another instant and very efficacious remedy is raking the beast, and drawing out the superabundant faeces.

(4) 'Another, in the first stage, frequently successful remedy, and always useful as an auxiliary, after the more prompt methods before recommended, is drenching.' For this purpose he mentions sweet oil, raw linseed or even train oil, or melted hog's lard.

"It was apparently thought by some people that hoven was due to a poison 'generated by plaistered clover,' i.e. after manuring with gypsum ..... 'The knife is the surest remedy for and antidote against this imaginary poison .....' "

This extract, together with the preceding review, illustrates how treatment of the ailment has progressed in the last 150 years.

SUMMARY

Recent research on bloat has placed emphasis on the widespread occurrence of foaming as an aetiological factor, though no figures are available to judge what percentage of cases are of this type. It is obvious that the elements involved in foaming bloat are complex, involving a number of plant and animal factors. The production of a stable foam may include contributions from saliva, bacteria and plant constituents, consisting of mucoproteins, polysaccharides, saponins and proteins, balanced with plant antifoaming agents.

Identification of the substances in the plant should permit the determination of the environmental and soil conditions concerned in the production of bloat-provoking pasture.

The use of antifoaming agents has shown considerable promise in the prevention and treatment of the condition, though the most economical control measures appear to be by suitable pasture management.

Insufficient knowledge of the acute condition prevents rational treatment of this state.

The cause of death remains unknown.

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